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THE TOXIC EFFECT OF CERTAIN CHEMICAL SOLUTIONS ON SPORES OF PENICILLIUM ITALICUM AND P. DIGITATUM[1,2]

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INTRODUCTION

THE BLUE AND THE GREEN molds (*Penicillium italicum* Wehmer and *P. digitatum* Sacc.) are the most common fungi causing soft decay in citrus fruits. They are world-wide in distribution, affecting fruits in orchards, in packing-houses, during transportation, and on the markets. In 1908, Powell (*27*)[5] reported that the losses from blue-mold decay in oranges during transportation from California were from $750,000 to $1,500,000 annually.

According to Sawada's (*34*) report in 1922, the two molds caused decay of oranges in Italy, the United States, Japan, and Formosa. Tindale (*38*) stated that in Victoria blue and green molds are the greatest enemies of oranges in cold storage and elsewhere. He (*39*) also reported that after two months' cold storage blue mold developed extensively. In 1928, Barker (*7*) stated that green mold causes serious losses in oranges from Spain, Palestine, Brazil, and the Argentine; less extensive damage is caused to oranges from South Africa, Australia, and California, and to grapefruit from Florida, Puerto Rico, and South Africa. In the same year Reichert and Littauer (*32*) reported that blue and green molds developed on picked fruit in Palestine. Bates (*8*) has shown that ship-

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[5] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

ment of early varieties in South Africa without precooling was attended by a very marked increase in mold wastage on discharge overseas. Yu (*43*) has reported that blue and green molds are found on all kinds of citrus fruits on the markets in China. Recently, Nattrass (*24*) reported that wastage of Cyprus oranges arriving in Europe was caused by blue and green molds.

In 1925, H. S. Fawcett (*13*) reported that of the decay of citrus fruits on arrival at eastern markets, 1.4 per cent was due to blue mold and 0.8 per cent was due to green mold. He also mentioned that the principal and almost the only kinds of decay found in California were the green mold and blue mold. In 1928, Barger (*4*) reported that Fawcett, after inspecting 500 field boxes of navel oranges in 8 Tulare County packing-houses in California in 1927, found that among the 1 per cent of rot in these oranges, 52 per cent of it was due to green mold, 32 per cent to a mixture of blue and green molds, 11 per cent to blue mold, and 5 per cent to other types of decay. According to Hopkins' report (*20*) for 1929, it was shown that the greatest losses during storage were due to *Penicillium italicum* and other fungi. Takeuchi (*37*) reported in 1929 that the rotting of satsuma oranges in storage or transit in Japan was caused by *P. italicum*, *P. digitatum*, and two other species of *Penicillium*. It was estimated that 89 per cent of the decayed fruits had *P. italicum* and 73 per cent had *P. digitatum*.

The prevention of any form of wounds is the most important means of reducing decay, but the use of solutions of certain substances has been tried out as a supplementary means to prevent the rot. A solution of borax was first tested and described by Fulton and Bowman (*17*) in 1924. Further suggestions and confirmations for its use have been reported by the following: Fulton and Winston (*18*), Barger and Hawkins (*6*), Barger (*4*), the Brogdex Company (*10*), Powell (*28*), Young and Read (*42*), Benton (*9*), Bates (*8*), Nattrass (*24*), Putterill (*30*), and by Winston (*41*). The solutions of soap and of borax used in citrus-fruit packing houses for washing and disinfecting have been reported by Fawcett (*14*) and by Shiver (*35*). Hodgson (*19*) claimed that the use of borax was rapidly going out of favor in the California citrus industry. Some workers (*8, 29*) have emphasized that borax treatment can be considered only as an adjunct to careful handling.

"Metbor," a new material said to equal borax as a decay preventive, was reported recently by Stewart (*36*) to have marked advantages over borax in regard to cold water solubility and other properties.

According to the Charter Oak House tests (*11*), the sodium hypochlorite process will prevent blue and green molds from developing in fruit

while it is in transit. Recently a stabilized sodium hypochlorite concentrate (*2*) has been manufactured; this contained 6 per cent sodium hypochlorite and when diluted according to directions was effective in controlling blue mold on apples and pears. Baker and Heald (*3*) found that rinsing apples for one minute with a sodium hypochlorite solution containing 0.4 per cent available chlorine was very effective in reducing the number of viable spores of *Penicillium expansum* on the surface and in the lenticels of apples and in decreasing losses from decay by this fungus.

Sodium bicarbonate and sodium carbonate are used in countries where borax treatment is prohibited by law. In 1928, Barger (*5*) first used sodium bicarbonate in controlling molds. The results were confirmed by Young and Read (*42*), by the Australian Citrus Preservation Committee (*1*), by Benton (*9*), by Bates (*8*), by Putterill and Davies (*31*), and by Putterill (*30*). Sodium carbonate was shown by Doidge (*12*) to be best for the control of *Penicillium* molds.

Tomkins and Trout (*40*) stated that storage of oranges in a humid atmosphere with ammonium carbonate or with crystals of ammonium carbonate reduces green-mold decay.

The study reported here was made for the purpose of securing more definite and effective means of controlling the blue and green molds by the use of chemical solutions.

MATERIALS USED

The original cultures of *Penicillium italicum* (No. 1746) and *P. digitatum* (No. 1438) were obtained from the stock cultures of the Division of Plant Pathology, Citrus Experiment Station, Riverside, California. The former was isolated by L. J. Klotz in 1930 from a decayed Valencia orange, and the latter by G. Savastano in 1927 from a decayed lemon. The medium used for these two fungi was 2 per cent glucose potato agar in the form of test-tube slants.

All cultures used throughout the experiments were incubated at 77° F (25° C), which was near the optimum temperature for growth on culture media as well as on orange fruits, as shown by Fawcett and Barger (*15*). The rate of sporulation of *Penicillium digitatum* is much slower than that of *P. italicum*. For the purpose of getting fair sporulations of these two fungi, *P. digitatum* was transferred to the slant 8 days earlier than *P. italicum*. For all treatments throughout the experiments, the age of *P. italicum* cultures used was 6 days and that of *P. digitatum* was 14 days.

The substances described below were used in solution form for treating both kinds of spores in the experiments:

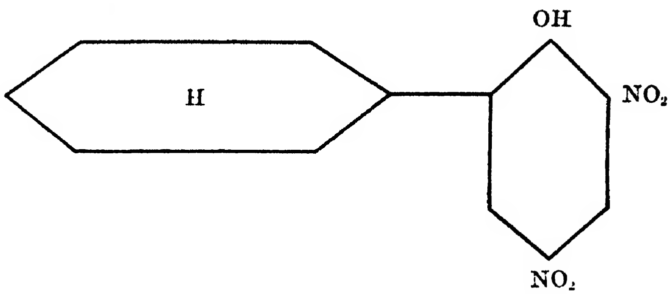
1. It was thought that a neutral soap of high purity might be used to facilitate wetting and to prevent clumping of spores. Accordingly a good grade of Castile soap was selected and tested in various concentrations.

2. Various concentrations of borax (sodium tetraborate decahydrate), $Na_2B_4O_7 \cdot 10H_2O$, were used. This preparation is also called sodium biborate or sodium pyroborate. It is a colorless monoclinic crystal or white powder and is slightly soluble in water and insoluble in alcohol.

3. A 6 per cent solution of a mixture of 2 parts borax and 1 part boric acid, H_3BO_3, was employed.

4. Metbor, which consists of 95 to 97 per cent sodium metaborate ($Na_2B_2O_4 \cdot H_2O$) and 3 to 5 per cent borax, was used in various concentrations.

5. Dinitro-o-cyclohexylphenol, is a yellow powder, having the empirical formula $C_{12}H_{14}N_2O_5$, and the structural formula:



It is only slightly soluble in distilled water, dissolving to the extent of 6.2 milligrams per liter of water. It was used in concentrations representing saturation, half saturation, and one-fourth saturation.

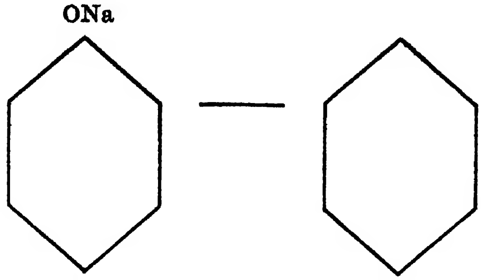
6. A 6 per cent sodium hypochlorite, NaOCl, was used as stock solution and then diluted to three concentrations of 1.0, 0.6, and 0.4 per cent.

7. Various concentrations of sodium bicarbonate, $NaHCO_3$, were used. This is also called acid sodium carbonate and baking soda. It is a white opaque powder or colorless crystals, soluble in water.

8. Sodium carbonate, Na_2CO_3 (anhydrous), was used in various concentrations. It is a white powder which is soluble in water. The form commonly used commercially is called soda ash.

9. A 0.4 per cent solution of chloramine-T or chlorazene, $CH_3C_6H_4SO_2$ $Na:NCl \cdot 3H_2O$ (sodium p-toluene-sulfo-chloramine), was also tested. It is also called mianine, actirin, tochlorine, chloroamine, tolamine, and Dakin's antiseptic. It takes the form of colorless crystals which are soluble in water.

10. Sodium o-phenylphenate was used as a 0.15 per cent solution. The chemical is represented by the formula:



It is a white powder which is difficultly soluble in water.

11. A 1 per cent solution of a commercial washing powder containing mostly soda ash (anhydrous sodium carbonate) with some caustic and a trace of pine oil, was also used.

METHODS

The methods described here have been used throughout the experiments as the standard procedure, unless otherwise stated. The more specific methods will be described under separate headings.

As soon as preparations for a certain experiment were completed the spore suspensions were made up in 0.25 per cent soap solution from agar slant cultures of *Penicillium italicum* and *P. digitatum*. About 10 minutes after shaking these suspensions, 5 cc of each of the suspensions was transferred with sterile pipettes to sterile centrifuge tubes. In order to precipitate the spores from suspension, these tubes were centrifuged for 3 minutes. Immediately after that the supernatant solution was decanted and into each tube was poured 10 cc of a desired concentration of the designated chemical. The tube was then shaken thoroughly. About 3 minutes before the desired treatment time had expired, the tubes were put in the centrifuge in order to precipitate the treated spores. At the end of 3 minutes, the treating solution was decanted and the spores were washed with sterile distilled water.

When experiments on the effect of various temperatures were conducted, treating solutions at the desired temperature were poured on the spores and the tubes immersed immediately in water baths at definite, controlled temperatures for a period of about 3 minutes less than the desired period of exposure. Then these tubes were put in the centrifuge which was fixed in an electric oven adjusted to the same temperature as the water bath. After the tubes were centrifuged for 3 minutes, the treat-

ing solution was decanted and the spores washed with sterile distilled water as before. The check tubes were secured after the spore suspensions in soap were centrifuged and decanted, by using 10 cc of sterile distilled water instead of the treating solution. At the end of the treatments, the number of spores per cubic centimeter was estimated by means of a Howard counting chamber. Generally, two counts for each fungus were made, one of the treated suspension and one of the untreated suspension. In order to determine the viability of the treated and untreated spores, germination and dilution plate tests in triplicate were conducted as follows.

Germination.—For the sake of obtaining accurate results in spore-germination tests, several factors mentioned by McCallan and Wilcoxon (*23*) were considered. They stated that the most important factors are: cleanliness of glassware, source and age of spores, density of spore suspension, germination medium, concentration of toxic agent, temperature, and time. For the germinations two Van Tieghem cells were sealed to each glass slide with vaseline. A drop of sterile distilled water was placed in each cell and a small amount of vaseline on the upper edge of each ring. A very small drop of fresh sweet-orange juice and one loopful (4 mm) of spore suspension were placed on a sterile cover glass which was then inverted over a cell. Then these cells were placed in the incubator at 77° F (25° C). After 24 hours of incubation, a drop of chloroform was introduced into one cell of a slide to stop the growth during the period of measurement; this was repeated in the other cell after 48 hours.

Dilution Method.—As soon as the hanging-drop germination tests were completed, dilutions of 1:10,000, 1:100,000, and 1:1,000,000 were made by means of sterile pipettes, and 9 cc and 99 cc water blanks. Each of the dilutions was transferred with a pipette to a sterile petri dish. Melted glucose potato agar was poured into these petri dishes, and was mixed and incubated at 77° F. After periods of 2 days and 3 days, the number of colonies in the dishes was counted.

All results shown in table 2 were recorded as an average of three tests for each experiment, unless otherwise noted. The average number of colonies per cubic centimeter in the dilution-plate counts was calculated by dividing the total number of colonies of the three dilutions by the decimal 0.000111, since dilutions of 1:10,000 (0.0001), 1:100,000 (0.00001), and 1:1,000,000 (0.000001) were used. The viability index was calculated by dividing the average number of colonies per cubic centimeter of dilution-plate counts by the average number of spores per cubic centimeter of the microscopic counts. In the last column of table 2 the viability of each fungus after each treatment is calculated on the

basis of an assumed value of 100 for the viability in the water check. This permits comparison of the two fungi in any given solution and gives at a glance the relative efficiency of the several treatments.

MEANS OF WETTING SPORES

Before starting the experiments on the effect of various chemical solutions, three different experiments were made with soap, the object being to determine the most suitable concentration for wetting spores and preventing clumping.

The Effect of Various Concentrations of Soap Solution.—Spore suspensions were made in five different concentrations of Castile soap, 10

TABLE 1

CONCENTRATION OF SOAP SOLUTION IN RELATION TO THE NUMBER OF SPORE CLUMPS AT ROOM TEMPERATURE

Fungus	Treatment		Average number of clumps per field*	Number of spores per cubic centimeter
	Per cent soap	Time, in minutes		
Penicillium italicum	0 10	10	4 8	3,827,157
	0 25	10	6.7	
	0 50	10	8.9	
	1 00	10	6.1	
	2 00	10	6 5	
	Check		9.6	
Penicillium digitatum	0.10	10	7.4	2,592,590
	0.25	10	3.1	
	0 50	10	4.8	
	1.00	10	6 8	
	2 00	10	3.4	
	Check		3.8	

* Average of 20 fields under the low power of the microscope.

cc of each being placed in sterile centrifuge tubes. These were centrifuged at room temperature for about 3 minutes, the total length of exposure being 5 minutes. Immediately after centrifuging, all the soap solutions were decanted and the tubes filled with 10 cc of sterile distilled water. The germination tests and plate method were then carried out as described above. The results are shown in table 2, entries 1 to 12.

Although none of these concentrations showed much inhibition of spore germination, the results (table 2) indicated that 0.25 per cent soap solution had the least effect on both spore germination and growth in petri dishes in the case of *Penicillium digitatum*.

TABLE 2

Toxicity of Various Chemical Solutions to the Conidia at Several Temperatures and Periods of Exposure

Entry No.	Fungus	Fungicide	Concentration of fungicide, per cent	pH of fungicide	Temperature during treatment, °F	Exposure, minutes	Average per cent germination		Average number of colonies per cc by dilution plate counts*	Average number of spores per cc by microscopic counts	Viability index†	Relative viability (water at 69° F = 100)
							After 24 hrs.	After 48 hrs.	A	B	A÷B	
1			0.10	9.73	69	5	98.9	100.0	2,801,801	3,950,614	0.709	77.3
2			0.25	9.90	69	5	99.5	100.0	3,108,108	3,950,614	0.787	85.8
3		Castile soap	0.50	9.88	69	5	99.1	100.0	3,576,576	3,950,614	0.905	98.6
4	P. italicum		1.00	9.97	69	5	98.6	100.0	4,279,279‡	3,950,614	1.083	118.1
5			2.00	10.05	69	5	99.5	100.0	5,189,189‡	3,950,614	1.313	143.1
6		Distilled water	Check	4.98	69	5	99.5	100.0	4,189,189	4,567,898	0.917	100.0
7			0.10	9.73	69	5	68.8	92.5	1,522,522	2,304,525	0.661	90.3
8			0.25	9.90	69	5	76.9	98.7	1,531,531	2,304,525	0.664	90.7
9		Castile soap	0.50	9.88	69	5	70.8	84.0	1,423,423	2,304,525	0.618	84.4
10	P. digitatum		1.00	9.97	69	5	72.8	100.0	1,270,270	2,304,525	0.551	75.2
11			2.00	10.05	69	5	71.5	82.5	1,324,324	2,304,525	0.575	78.5
12		Distilled water	Check	4.98	69	5	76.6	92.5	1,837,837	2,510,289	0.732	100.0
13			0.10, 6¶	9.30	69	10, 5¶	52.2	93.4	2,909,909	3,497,939	0.832	71.5
14			0.25; 6	9.30	69	10, 5	46.6	99.2	2,765,765	3,497,939	0.791	68.0
15		Castile soap, borax	0.50; 6	9.30	69	10, 5	29.1	91.9	3,090,090	3,497,939	0.883	75.9
16			1.00; 6	9.30	69	10; 5	32.3	75.8	4,189,189‡	3,497,939	1.198	103.0
17	P. italicum		2.00, 6	9.30	69	10, 5	31.8	96.4	4,171,171‡	3,497,939	1.192	102.5
18			0.00; 6	9.30	69	5	41.4	98.7	3,090,090	3,497,939	0.883	75.9
19		Distilled water	Check	4.98	69	5	96.2	100.0	5,981,981‡	5,144,022	1.163	100.0
20			0.10, 6	9.30	69	10, 5	18.8	28.1	1,000,000	2,716,047	0.368	62.6
21			0.25, 6	9.30	69	10; 5	13.8	54.3	846,846	2,716,047	0.312	53.1
22		Castile soap; borax	0.50; 6	9.30	69	10; 5	4.1	36.5	936,936	2,716,047	0.345	58.7
23			1.00; 6	9.30	69	10; 5	22.7	75.5	684,684	2,716,047	0.252	42.9
24	P. digitatum		2.00; 6	9.30	69	10, 5	43.6	74.9	990,990	2,716,047	0.365	62.1
25			0.00; 6	9.30	69	5	59.2	93.6	1,045,045	2,716,047	0.385	65.5
26		Distilled water	Check	4.98	69	5	97.7	100.0	1,765,765	3,004,113	0.588	100.0

27				4.98	69	5	99.6	100.0	5,108,828	5,226,333	0.977	100.0
28	*P. italicum*	Distilled water		5.04	100	5	97.2	100.0	1,846,846	4,444,107	0.415	42.5
29				5.06	110	5	58.6	98.4	747,747	4,444,107	0.168	17.2
30				5.30	120	5	0.8	11.5	9,009	4,444,107	0.002	0.2
31				4.98	69	5	99.2	100.0	1,504,504	3,168,721	0.475	100.0
32	*P. digitatum*	Distilled water		5.04	100	5	95.8	99.6	792,792	2,921,808	0.271	57.1
33				5.06	110	5	59.2	99.6	509,509	2,921,808	0.174	36.6
34				5.30	120	5	0.4	8.2	9,009	2,921,808	0.003	0.6
35			6	8.98	110	2	65.5	100.0	3,666,666	8,768,759	0.418	88.4
36			6	8.98	110	4	80.0	100.0	2,882,882	8,768,759	0.329	69.6
37			6	8.98	110	6	13.6	81.6	2,774,774	8,768,759	0.316	66.8
38		Borax	6	8.98	110	8	13.1	57.0	1,549,549	8,768,759	0.177	37.4
39			6	8.98	110	10	1.2	24.4	531,531	8,768,759	0.061	12.9
40	*P. italicum*		6	8.98	110	12	2.4	39.9	792,792	8,768,759	0.090	19.0
41			6	8.98	110	14	0.1	24.3	441,441	8,768,759	0.050	10.6
42			6	8.98	110	16	0.1	22.1	230,630	8,768,759	0.026	5.5
43		Distilled water	Check	4.98	69	5	100.0	100.0	4,441,441	9,386,042	0.473	100.0
44			6	8.98	110	2	68.1	99.1	1,171,171	3,580,177	0.327	60.8
45			6	8.98	110	4	82.6	99.4	837,837	3,580,177	0.234	43.5
46			6	8.98	110	6	32.1	64.2	864,864	3,580,177	0.247	45.9
47		Borax	6	8.98	110	8	3.6	12.4	504,504	3,580,177	0.141	26.2
48			6	8.98	110	10	3.9	33.4	301,801	3,580,177	0.084	15.6
49	*P. digitatum*		6	8.98	110	12	3.6	27.9	141,141	3,580,177	0.039	7.2
50			6	8.98	110	14	1.0	6.5	193,693	3,580,177	0.054	10.0
51			6	8.98	110	16	0.9	4.9	128,828	3,580,177	0.036	6.7
52		Distilled water	Check	4.98	69	5	99.8	100.0	2,306,306	4,283,165	0.538	100.0

* Total number of colonies in three dilution plates *divided by* 0.000111.

† Average number of colonies per cubic centimeter determined by dilution plate count *divided by* the average number of spores per cubic centimeter determined by microscopic count *equals* the viability index.

‡ Theoretically, this figure should not have exceeded the microscopic count.

¶ In this column in entries 13 to 26 the first number refers to castile soap and the number following the semicolon refers to borax.

TABLE 2—(*Continued*)

Entry No.	Fungus	Fungicide	Concentration of fungicide, per cent	pH of fungicide	Temperature during treatment, °F	Exposure, minutes	Average per cent germination: After 24 hrs.	Average per cent germination: After 48 hrs.	Average number of colonies per cc by dilution plate counts* A	Average number of spores per cc by microscopic counts B	Viability index† A+B	Relative viability (water at 69° F=100)
53			6	9.30	69	5	49 0	78 6	3,162,162	4,691,354	0.674	62.1
54			6	9.14	80	5	40.9	73.2	4,108,108	4,691,354	0.876	80.7
55		Borax	6	9.00	100	5	36.5	40.9	3,261,261	4,691,354	0.695	64.0
56	*P. italicum*		6	8 96	110	5	12.9	40.8	1,567,567	4,691,354	0 334	30 8
57			6	8 93	120	5	0.1	2.3	0	4,691,354	0.000	0.0
58		Distilled water	Check	4.98	69	5	100.0	100.0	5,720,720‡	5,267,419	1.086	100.0
59			6	9.30	69	5	51.5	77.1	1,207,207	3,786,005	0.319	56.8
60			6	9.14	80	5	55.7	62.3	1,297,297	3,786,005	0.343	61.0
61		Borax	6	9.00	100	5	39.7	58.2	1,027,027	3,786,005	0.271	48.2
62	*P. digitatum*		6	8.96	110	5	5.2	53.5	585,585	3,786,005	0.155	27.6
63			6	8.93	120	5	1.0	1 3	2,702	3,786,005	0.001	0.1
64		Distilled water	Check	4.98	69	5	99.0	100.0	2,450,450	4,362,136	0.562	100.0
65			4	8.95	110	5	13 7	85.9	1,729,729	5,843,620	0.296	31.1
66			6	8 98	110	5	6.0	41.6	1,324,324	5,843,620	0.227	23.9
67		Borax	8	9 03	110	5	0.8	23 7	1,387,387	5,843,620	0.237	24.9
68	*P. italicum*		10	9 08	110	5	0.6	7.9	1,144,144	5,843,620	0.196	20.6
69			12	9.11	110	5	0.0	1.7	936,936	5,843,620	0.160	16.8
70		Distilled water	Check	4.98	69	5	100.0	100.0	6,621,621	6,962,546	0.951	100.0
71			4	8.95	110	5	59.4	89.2	1,297,297	4,444,441	0.292	76.2
72			6	8 98	110	5	5.7	51.0	1,045,045	4,444,441	0.235	61.4
73		Borax	8	9 03	110	5	4.1	39.4	909,909	4,444,441	0.205	53.5
74	*P. digitatum*		10	9.08	110	5	2.2	31.9	711,711	4,444,441	0.160	41.8
75			12	9.11	110	5	0.0	1.3	401,801	4,444,441	0.090	23.5
76		Distilled water	Check	4.98	69	5	100.0	100.0	1,891,891	4,938,268	0.383	100.0

77			6	8.18	100	5	59.6	99.6	2,342,342	4,067,771	0.576	56.7
78		Borax-boric acid	6	8.15	110	5	0.0	40.8	743,243	4,067,771	0.183	18.0
79	*P. italicum*		6	8.14	120	5	0.0	11.9	7,207	4,067,771	0.002	0.2
80		Distilled water	Check	4.98	69	5	100.0	100.0	5,225,225‡	5,144,029	1.016	100.0
81			6	8.18	100	5	90.8	100.0	1,153,153	2,757,199	0.418	94.1
82		Borax-boric acid	6	8.15	110	5	4.6	59.9	486,486	2,757,199	0.176	39.6
83	*P. digitatum*		6	8.14	120	5	0.0	0.0	2,702	2,757,199	0.001	0.2
84		Distilled water	Check	4.98	69	5	99.9	100.0	1,369,369	3,086,084	0.444	100.0
85			6	10.18	69	2	99.2	100.0	2,261,261	3,621,396	0.624	61.7
86		Metbor	6	10.18	69	5	91.8	100.0	2,216,216	3,621,396	0.612	60.5
87	*P. italicum*		6	10.18	69	10	87.7	99.5	1,882,882	3,621,396	0.520	51.4
88			6	10.18	69	15	65.8	91.7	1,756,756	3,621,396	0.485	48.0
89		Distilled water	Check	4.98		5	99.6	100.0	4,036,036‡	3,991,432	1.011	100.0
90			6	10.18	69	2	97.4	100.0	1,261,261	3,004,112	0.420	89.9
91		Metbor	6	10.18	69	5	96.9	100.0	1,099,099	3,004,112	0.366	78.4
92	*P. digitatum*		6	10.18	69	10	77.6	98.4	981,981	3,004,112	0.327	70.0
93			6	10.18	69	15	49.6	79.2	900,900	3,004,112	0.300	64.2
94		Distilled water	Check	4.98	69	5	99.8	100.0	1,576,576	3,374,482	0.467	100.0
95			6	10.18	69	5	91.8	100.0	2,216,216	3,621,396	0.612	62.6
96		Metbor	6	9.95	100	5	87.1	99.8	2,198,198	4,444,107	0.495	50.7
97	*P. italicum*		6	9.86	110	5	78.1	99.2	1,981,981	4,444,107	0.446	45.6
98			6	9.79	120	5	0.0	0.6	23,423	4,444,107	0.005	0.5
99		Distilled water	Check	4.98	69	5	99.6	100.0	5,108,108	5,226,333	0.977	100.0
100			6	10.18	69	5	96.9	100.0	1,000,000	3,004,112	0.333	70.1
101		Metbor	6	9.95	100	5	59.5	98.0	639,639	2,921,808	0.219	46.1
102	*P. digitatum* ..		6	9.86	110	5	36.3	91.0	630,630	2,921,808	0.216	45.5
103			6	9.79	120	5	0.0	0.4	2,702	2,921,808	0.001	0.2
104		Distilled water .	Check	4.98	69	5	99.2	100.0	1,504,504	3,168,721	0.475	100.0

* Total number of colonies in three dilution plates *divided by* 0.000111.

† Average number of colonies per cubic centimeter determined by dilution plate count *divided by* the average number of spores per cubic centimeter determined by microscopic count *equals* the viability index.

‡ Theoretically, this figure should not have exceeded the microscopic count.

TABLE 2—(*Continued*)

Entry No.	Fungus	Fungicide	Concentration of fungicide, per cent	pH of fungicide	Temperature during treatment, °F	Exposure, minutes	Average per cent germination After 24 hrs.	Average per cent germination After 48 hrs.	Average number of colonies per cc by dilution plate counts* *A*	Average number of spores per cc by microscopic counts *B*	Viability index† *A*÷*B*	Relative viability (water at 69° F=100)
105	*P. italicum*	Metbor	4	10.07	69	5	78.0	94.5	2,171,171	3,621,396	0.599	59.2
106	*P. italicum*	Metbor	6	10.18	69	5	91.8	100.0	2,216,216	3,621,396	0.612	60.5
107	*P. italicum*	Metbor	8	10.22	69	5	73.2	91.2	1,909,909	3,621,396	0.527	52.1
108	*P. italicum*	Metbor	10	10.27	69	5	44.3	76.8	1,900,900	3,621,396	0.525	51.9
109	*P. italicum*	Metbor	12	10.31	69	5	31.9	60.2	1,864,864	3,621,396	0.515	50.9
110	*P. italicum*	Distilled water	Check	4.98	69	5	99.6	100.0	4,036,036‡	3,991,432	1.011	100.0
111	*P. digitatum*	Metbor	4	10.07	69	5	97.8	99.0	1,198,198	3,004,112	0.399	85.4
112	*P. digitatum*	Metbor	6	10.18	69	5	96.9	100.0	1,099,099	3,004,112	0.366	78.4
113	*P. digitatum*	Metbor	8	10.22	69	5	78.2	92.5	918,918	3,004,112	0.306	65.5
114	*P. digitatum*	Metbor	10	10.27	69	5	56.5	83.8	576,576	3,004,112	0.192	41.1
115	*P. digitatum*	Metbor	12	10.31	69	5	35.4	65.4	441,441	3,004,112	0.147	31.5
116	*P. digitatum*	Distilled water	Check	4.98	69	5	99.8	100.0	1,576,576	3,374,482	0.467	100.0
117	*P. italicum*	Dinitro-o-cyclohexylphenol	0.001	5.35	69	2	92.2	99.6	1,189,189	2,551,438	0.466	47.6
118	*P. italicum*	Dinitro-o-cyclohexylphenol	0.0005	5.16	69	2	91.5	100.0	1,162,162	2,551,438	0.455	46.5
119	*P. italicum*	Dinitro-o-cyclohexylphenol	0.00025	4.96	69	2	91.7	100.0	954,954	2,551,438	0.374	38.2
120	*P. italicum*	Distilled water	Check	4.98	69	5	99.5	100.0	5,117,117	5,226,333	0.979	100.0
121	*P. digitatum*	Dinitro-o-cyclohexylphenol	0.001	5.35	69	2	88.0	100.0	864,864	2,181,068	0.396	88.2
122	*P. digitatum*	Dinitro-o-cyclohexylphenol	0.0005	5.16	69	2	94.4	100.0	1,018,018	2,181,068	0.466	103.8
123	*P. digitatum*	Dinitro-o-cyclohexylphenol	0.00025	4.96	69	2	95.0	100.0	1,063,063	2,181,068	0.487	108.5
124	*P. digitatum*	Distilled water	Check	4.98	69	5	99.7	100.0	1,459,459	3,251,026	0.449	100.0
125	*P. italicum*	NaOCl	0.4	11.10	69	2	0.0	0.0	0	7,736,619	0.000	0.0
126	*P. italicum*	NaOCl	0.6	11.14	69	2	0.8	2.0	0	7,736,619	0.000	0.0
127	*P. italicum*	NaOCl	1.0	11.14	69	2	0.0	0.0	0	7,736,619	0.000	0.0
128	*P. italicum*	Distilled water	Check	4.98	69	5	99.4	100.0	6,369,369	11,275,711	0.565	100.0

129		NaOCl	0.4	11.10	69	2	0.0	0.0	0	2,716,047	0.000	0.0
130			0.6	11.14	69	2	0.0	0.0	0	2,716,047	0.000	0.0
131	*P. digitatum*		1.0	11.14	69	2	0.0	0.0	0	2,716,047	0.000	0.0
132		Distilled water	Check	4.98	69	5	99.0	100.0	1,387,387	3,415,635	0.406	100.0
133		$NaHCO_3$	6	7.96	86	2	99.8	100.0	4,801,801	5,802,465	0.827	73.3
134			6	7.96	86	5	99.7	100.0	4,657,657	5,802,465	0.803	71.2
135	*P. italicum*		6	7.96	86	10	99.6	100.0	4,459,459	5,802,465	0.768	68.1
136		Distilled water	Check	4.98	69	5	99.7	100.0	7,522,522‡	6,666,661	1.128	100.0
137		$NaHCO_3$	6	7.96	86	2	99.7	100.0	1,171,171	3,086,417	0.379	62.9
138			6	7.96	86	5	99.6	100.0	1,261,261	3,086,417	0.409	67.8
139	*P. digitatum*		6	7.96	86	10	97.4	100.0	1,072,072	3,086,417	0.347	57.5
140		Distilled water	Check	4.98	69	5	99.8	100.0	2,009,009	3,333,330	0.603	100.0
141		$NaHCO_3$	6	7.96	86	5	99.7	100.0	4,657,657	5,802,465	0.803	69.2
142			6	7.85	100	5	99.2	100.0	2,540,540	4,444,437	0.572	49.3
143			6	7.85	110	5	96.9	100.0	2,162,162	4,444,437	0.486	41.9
144			6	7.82	120	5	0.0	25.2	18,018	4,444,437	0.004	0.3
145	*P. italicum*	Na_2CO_3	6	10.16	86	5	48.3	98.1	747,747	4,067,771	0.184	15.8
146			6	9.83	100	5	39.4	93.9	365,765	4,444,437	0.082	7.1
147			6	9.74	110	5	1.4	33.3	28,828	4,444,437	0.006	0.5
148			6	9.64	120	5	0.0	0.0	0	4,444,437	0.000	0.0
149		Distilled water	Check	4.98	69	5	100.0	100.0	5,540,540‡	4,773,659	1.161	100.0
150		$NaHCO_3$	6	7.96	86	5	99.8	100.0	1,261,261	3,086,417	0.409	78.4
151			6	7.85	100	5	99.2	100.0	1,351,351	2,921,808	0.462	88.5
152			6	7.85	110	5	98.5	100.0	1,153,153	2,921,808	0.395	75.7
153			6	7.82	120	5	0.5	11.8	23,423	2,921,808	0.008	1.5
154	*P. digitatum*	Na_2CO_3	6	10.16	86	5	61.5	91.8	459,459	2,757,199	0.166	31.8
155			6	9.83	100	5	44.7	78.0	450,450	2,921,808	0.154	29.5
156			6	9.74	110	5	0.4	6.0	28,828	2,921,808	0.010	1.9
157			6	9.64	120	5	0.0	0.0	0	2,921,808	0.000	0.0
158		Distilled water	Check	4.98	69	5	99.6	100.0	1,738,738	3,333,330	0.522	100.0

* Total number of colonies in three dilution plates *divided by* 0.000111.

† Average number of colonies per cubic centimeter determined by dilution plate count *divided by* the average number of spores per cubic centimeter determined by microscopic count *equals* the viability index.

‡ Theoretically, this figure should not have exceeded the microscopic count

TABLE 2—(*Concluded*)

Entry No.	Fungus	Fungicide	Concentration of fungicide, per cent	pH of fungicide	Temperature during treatment, °F	Exposure, minutes	Average per cent germination: After 24 hrs.	Average per cent germination: After 48 hrs.	Average number of colonies per cc by dilution plate counts* A	Average number of spores per cc by microscopic counts B	Viability index† A÷B	Relative viability (water at 69° F=100)
159	*P. italicum*	NaHCO$_3$	2	8.30	86	5	99 6	100 0	5,243,243	5,802,465	0.904	92.4
160		NaHCO$_3$	6	8 13	86	5	99.7	100.0	4,657,657	5,802,465	0.803	82.1
161		NaHCO$_3$	10	8 00	86	5	100 0	100.0	4.657,657	5,802,465	0.803	82.1
162		Distilled water	Check	4 98	69	5	99.7	100 0	6,522,522	6,666,661	0 978	100.0
163	*P. digitatum*	NaHCO$_3$	2	8 30	86	5	99.1	100.0	1,729,729	3,086,417	0.560	92.9
164		NaHCO$_3$	6	8 13	86	5	99.8	100 0	1.261,261	3,086,417	0.409	67.8
165		NaHCO$_3$	10	8 00	86	5	85.1	100.0	1,189,189	3,086,417	0.385	63.8
166		Distilled water	Check	4.98	69	5	99.8	100.0	2,009,009	3,333,330	0.603	100.0
167	*P. italicum*	Na$_2$CO$_3$	6	10 16	86	2	94.6	100.0	810,810	4,067,771	0.199	19.6
168		Na$_2$CO$_3$	6	10 16	86	5	48.3	98.1	747.747	4.067,771	0.184	18.1
169		Na$_2$CO$_3$	6	10.16	86	10	32.4	83.6	558,558	4,067,771	0.137	13.5
170		Distilled water	Check	4.98	69	5	100.0	100.0	5,225,225‡	5,144,029	1.016	100.0
171	*P. digitatum*	Na$_2$CO$_3$	6	10.16	86	2	70.7	100.0	657,657	2,757,199	0.238	53.6
172		Na$_2$CO$_3$	6	10.16	86	5	61 5	91.8	459,459	2,757,199	0.167	37.6
173		Na$_2$CO$_3$	6	10.16	86	10	30.9	63.4	324.324	2,757,199	0.118	26.6
174		Distilled water	Check	4 98	69	5	99.9	100.0	1,369,369	3,086,084	0.444	100.0
175	*P. italicum*	Na$_2$CO$_3$	2	10 20	86	5	99.0	100.0	2,837,837	4,067,771	0.698	68.7
176		Na$_2$CO$_3$	6	10 15	86	5	48.3	98.1	747,747	4,067,771	0.184	18.1
177		Na$_2$CO$_3$	10	9 90	86	5	39.4	72.9	209,903	4,067,771	0.052	5.1
178		Distilled water	Check	4.98	69	5	100.0	100.0	5,225,225‡	5,144,029	1.016	100.0
179	*P. digitatum*	Na$_2$CO$_3$	2	10.20	86	5	99.4	100.0	1,036,036	2,757,199	0.376	84.7
180		Na$_2$CO$_3$	6	10.15	86	5	61.5	91.8	459,459	2,757,199	0.167	37.6
181		Na$_2$CO$_3$	10	9.90	86	5	28.3	64.9	243,243	2,757,199	0.088	19.8
182		Distilled water	Check	4.98	69	5	68.9	100.0	1,369,369	3,086,084	0.444	100.0

183	*P. italicum*	Chloramine-T	0.4	6.52	69	5	87.1	98.5	873,873	4,444,107	0.197	24.5
184			0.4	6.47	100	5	94.4	100.0	711,711	2,592,590	0.274	34.1
185			0.4	6.45	110	5	0.0	78.5	9,009	2,592,590	0.003	0.4
186			0.4	6.55	120	5	0.0	0.0	0	2,592,590	0.000	0.0
187		Distilled water	Check	4.98	69	5	99.2	100.0	2,774,774	3,455,787	0.803	100.0
188	*P. digitatum*	Chloramine-T	0.4	6.52	69	5	97.9	100.0	1,108,108	2,921,808	0.379	92.0
189			0.4	6.47	100	5	97.6	100.0	1,045,045	2,716,041	0.385	93.4
190			0.4	6.45	110	5	20.8	97.1	234,234	2,716,041	0.086	20.9
191			0.4	6.55	120	5	0.0	0.0	0	2,716,041	0.000	0.0
192		Distilled water	Check	4.98	69	5	99.2	100.0	1,270,270	3,086,417	0.412	100.0
193	*P. italicum*	Sod. o-phenylphenate	0.15	9.67	69	5	42.4	98.6	1,468,468	4,444,107	0.330	33.8
194		Chloramine-T	0.40	6.52	69	5	87.1	98.5	873,873	4,444,107	0.197	20.2
195		Washing powder	1.00	9.90	69	5	98.1	100.0	2,648,648	4,444,107	0.596	61.0
196		Distilled water	Check	4.98	69	5	99.6	100.0	5,108,108	5,226,333	0.977	100.0
197	*P. digitatum*	Sod. o-phenylphenate	0.15	9.67	69	5	5.2	63.6	128,828	2,921,808	0.044	9.3
198		Chloramine-T	0.40	6.52	69	5	97.9	100.0	1,108,108	2,921,808	0.379	79.8
199		Washing powder	1.00	9.90	69	5	98.5	100.0	1,153,153	2,921,808	0.395	83.2
200		Distilled water	Check	4.98	69	5	99.2	100.0	1,504,504	3,168,721	0.475	100.0

* Total number of colonies in three dilution plates *divided by* 0.000111.

† Average number of colonies per cubic centimeter determined by dilution plate count *divided by* the average number of spores per cubic centimeter determined by microscopic count *equals* the viability index.

‡ Theoretically, this figure should not have exceeded the microscopic count.

The Concentrations of Soap Solution in Relation to Number of Spore Clumps.—Five concentrations of Castile soap solution were used to determine the best concentration for dispersing spores of the two molds. Spore suspensions were made with sterile, distilled water; each of 6 centrifuge tubes was then filled with 5 cc of the suspension. After centrifuging for 3 minutes, the supernatant water was decanted and the tubes were filled, respectively, with 10 cc of the above five concentrations of soap solution; the check tubes were filled with sterile, distilled water. Ten minutes after the solutions had been added, the spore clumps were counted by means of a Howard counting chamber under the low power of the microscope. The results are shown in table 1 (p. 7).

Table 1 shows that 0.25 per cent soap solution is the best concentration for dispersing spores of *Penicillium digitatum,* that is, fewer clumps appeared when this concentration was used. This is in harmony with the results on the effect of various concentrations of soap solution on spore germination and on the growth in petri dishes. In the case of *P. italicum* the fewest clumps appeared in the 0.10 per cent solution. Because of the indications of these experiments and because the several soap concentrations were all relatively nontoxic, a concentration of 0.25 per cent was chosen for use with both fungi in all experiments subsequent to the third (entries 35 *et seq.* of table 2).

Killing Effect of Borax in Relation to Concentration of Soap Solution.—In comparing the effect of the concentration of soap solution on the killing effect of borax, the following procedures were followed: First, treatment was made with different concentrations of soap solution for 10 minutes. This was followed by immersion in a 6 per cent borax solution for 5 minutes at room temperature (66° to 72°, average 69° F). Secondly, treatment was made with 6 per cent borax solution but without any previous treatment with soap solution. Finally, for checks, the spores were not subjected to borax treatment. Results are given in table 2, entries 13 to 26.

According to the results, a 6 per cent borax solution is more effective in checking the spore germination and the plate growth of both fungi when used in conjunction with concentrations of 0.1 to 1.0 per cent soap solutions.

From the results of the three experiments discussed above, it is also seen that 0.25 per cent soap solution is suitable for wetting spores of these fungi. Hereafter, unless otherwise noted, the spores for all the experiments were first wetted with this concentration of soap before any further tests were made.

EFFECT OF TEMPERATURE ON SPORES IN DISTILLED WATER

Experiments were conducted to find how different temperatures affected the spores of the two molds. Winston (*41*) had reported that water at 110° F or above showed effective control of decay.

The general procedure for this experiment was the same as stated above, except that special attention was paid to obtaining and maintaining the desired temperatures. The more important steps for this experiment may be described briefly as follows: When the spore suspensions were centrifuged and the supernatant soap solution was poured off, distilled water which had been previously heated to the desired temperature was poured into the centrifuge tube and shaken thoroughly. Immediately after that, the tubes were put in the water bath for about 2 minutes and then into the centrifuge, which had been placed in an electric oven at the same temperature. After having been centrifuged for about 3 minutes, the tubes were removed and the warm water replaced with sterile distilled water at room temperature. Germination and plating tests were then made; the results are reported in table 2, entries 27 to 34.

The data show that the percentage of germination of the spores as measured by direct counts or plate cultures decreases as the temperatures increase. At 120° F, germination and plate growth are greatly repressed.

EFFECT OF BORAX

The preliminary results of the experiments of Fulton and Bowman (*17*) have shown that a commercial borax solution of 5 per cent or 10 per cent greatly reduces blue-mold rot of citrus fruits under experimental conditions. Further experiments by Fulton and Winston (*18*) suggest the use of the 5 per cent borax solution at 120° F for 5 minutes. This procedure was later supported and patented by the Brogdex Company (*10*). The experiments by Barger and Hawkins (*6*) at first indicated that 2.5 per cent boric acid at 120° F gave very promising results in controlling blue mold (*Penicillium italicum*). Later borax was tested and found to be as effective as boric acid and was much cheaper than the latter. From the results based on experimental data and data obtained from the commercial shipments, Barger (*4*) concluded that both blue- or green-mold decay can be controlled by 7 per cent borax solution at 110° F for 5 minutes. This has been confirmed by Reichert and Littauer (*33*). As a result of experiments in South Africa, Powell (*28*) suggests the use of a hot 2.5

per cent borax solution, or boric acid, or mixtures of both for the control of the green mold (*P. digitatum*). Benton (*9*) found that a 4-minute immersion in 8 per cent borax solution at 110° F was effective in preventing decay in oranges. By the results of tests, Putterill (*30*) supports the use of 8 per cent borax. He also mentions that 4 per cent borax and 4 per

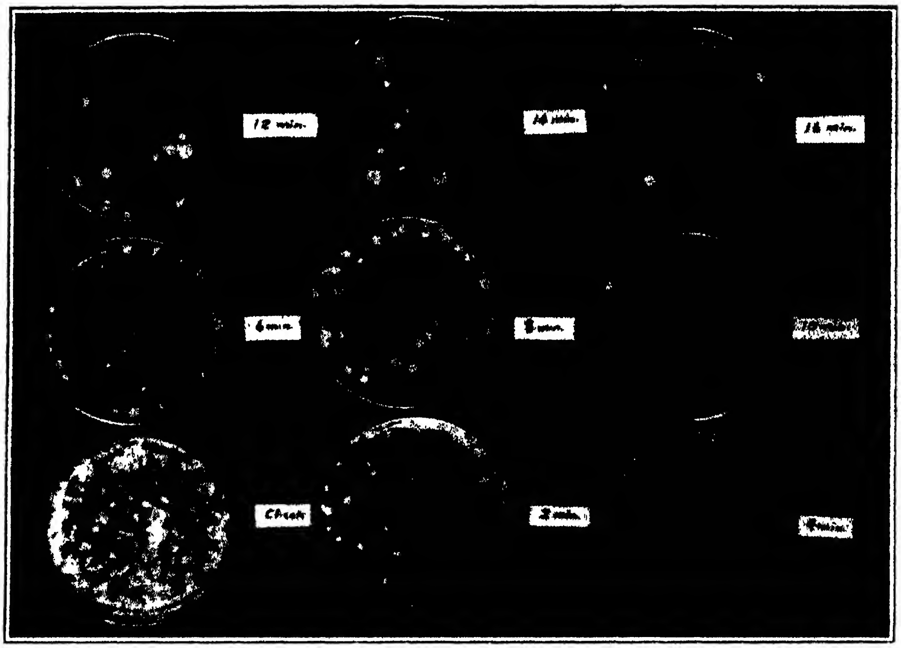


Fig. 1.—Effect on spores of *Penicillium italicum* exposed to 6 per cent borax solution at 110° F for various lengths of time. The plates represent a 1:100,000 dilution of the spore suspensions. The untreated suspension yielded numerous colonies, while treatments of 10 minutes or longer killed most of the spores.

cent sodium bicarbonate are of equal effectiveness at high temperatures. but the former is less effective at lower temperatures.

In order to determine the effect of the interrelation of concentrations of borax solution, with the length of treatment and various temperatures on the spores of both *Penicillium italicum* and *P. digitatum*, the following three experiments were conducted.

Time of Exposure.—In each series of 16 tests, 6 per cent borax solution at 110° F (43° C) was used for 2, 4, 6, 8, 10, 12, 14, and 16 minutes; as checks, 2 of the tests were made without chemical treatment except in 0.25 per cent soap solution. The results are given in table 2, entries 35 to 52, and shown in figure 1.

The results show that the percentage of spore germination of *Penicillium italicum* in 6 per cent borax at 110° F for 16 minutes is 0.1, but

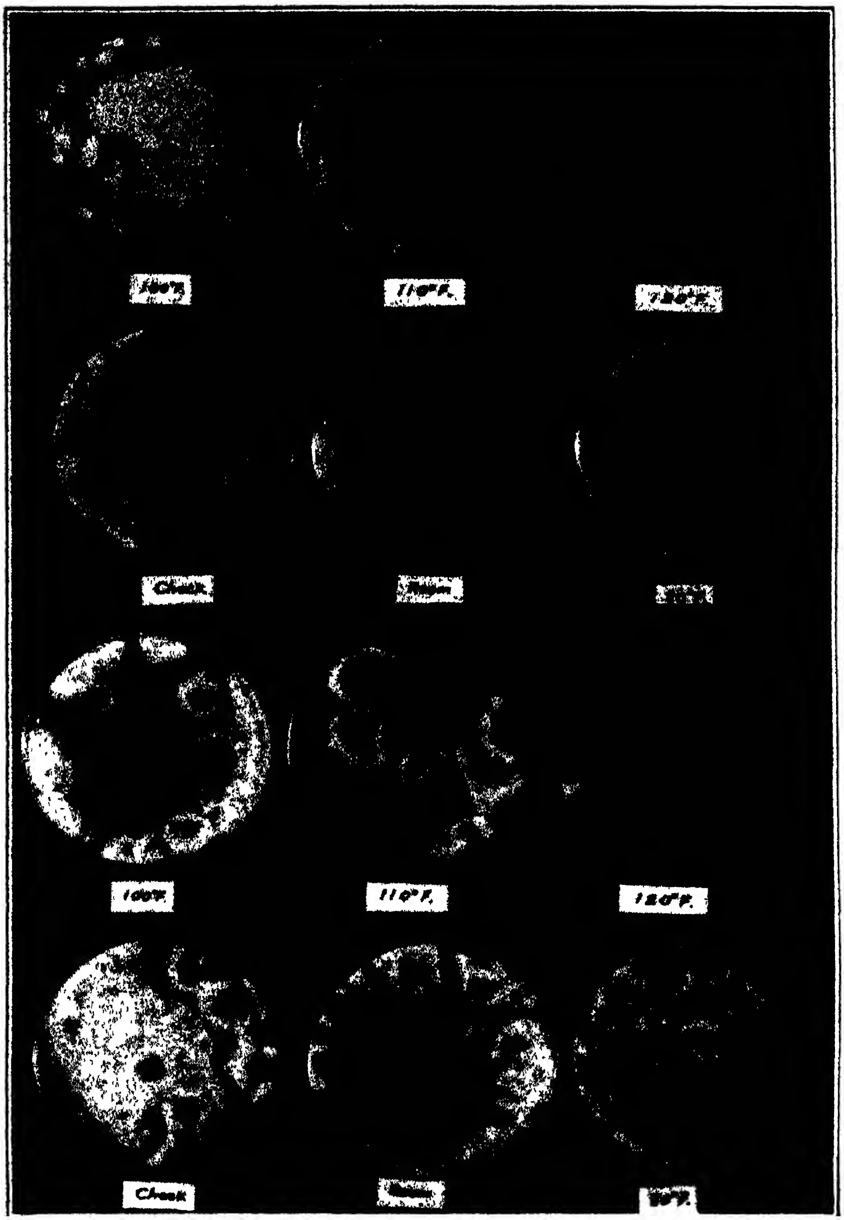


Fig. 2.—Effect of 6 per cent borax solution at various temperatures (from room temperature to 120° F), on spores of *Penicillium italicum* (upper 6 plates), and on *P. digitatum* (lower 6 plates); exposure time, 5 minutes. The plates represent a 1:100,000 dilution of the spore suspensions. The untreated spore suspensions of the two species at temperatures below 100° F yielded numerous colonies, while treatments at 120° F killed all the spores of *P. italicum* and nearly all those of *P. digitatum*.

at the same temperature for 10 minutes it is 1.2. Similar relations were found with *P. digitatum;* that is, 0.9 per cent of the conidia germinated after 110° F for 16 minutes, 3.9 per cent germinated after 110° F for 10 minutes. The results indicate that the longer the time of exposure to 6 per cent borax at the same temperature (110° F), the more effective

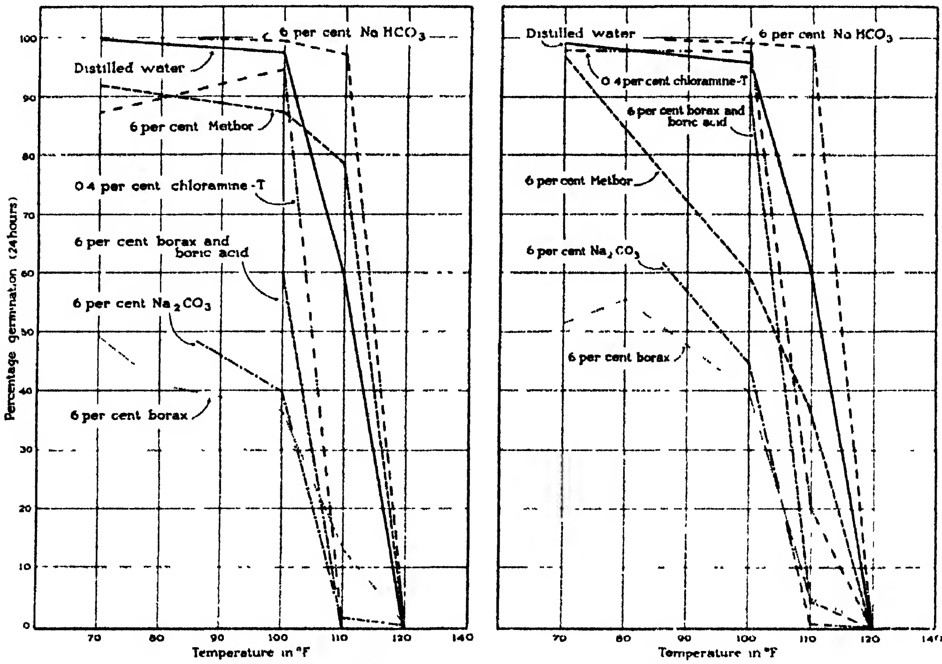


Fig. 3.—Left: Comparative effect of six solutions and distilled water at various temperatures, on the spore germination of *Penicillium italicum;* exposure time, 5 minutes. Six per cent borax-boric acid and 0.4 per cent chloramine-T at 110° F killed all the spores while 6 per cent sodium carbonate killed nearly all.

Right: Effect of the same solutions on the spore germination of *P. digitatum.* Six per cent solutions of sodium carbonate, borax-boric acid, and borax at 110° F killed nearly all the spores.

For convenience in plotting, the graphs are shown as starting at 70° F instead of 69° as shown in the tables; that temperature is still within the room-temperature range of 66°–72°, although not the exact average.

is the material in reducing both spore germination and the growth in plates of the two fungi.

Temperature Relations.—In order to determine whether the toxicity of borax is affected by higher temperatures, spores of both fungi were treated with 6 per cent borax solution for 5 minutes at the following temperatures: 66° to 72° (room temperature), 80°, 100°, 110°, and 120° F. The results are summarized in table 2, entries 53 to 64, and illustrated in figure 2.

The results show that, when the temperature increased from room tem-

perature to 120° F (49° C), the spore germination (24 hours later) decreased from 49.0 per cent to 0.1 per cent in the case of ***Penicillium italicum***. In the case of ***P. digitatum***, the same tendency is found, that is, germination decreased from 51.5 per cent to 1.0 per cent.

However, even in distilled water the higher temperatures are effective in decreasing germination (table 2, entries 27 to 34), though 6 per cent borax at these temperatures is seen to be even more effective, indicating

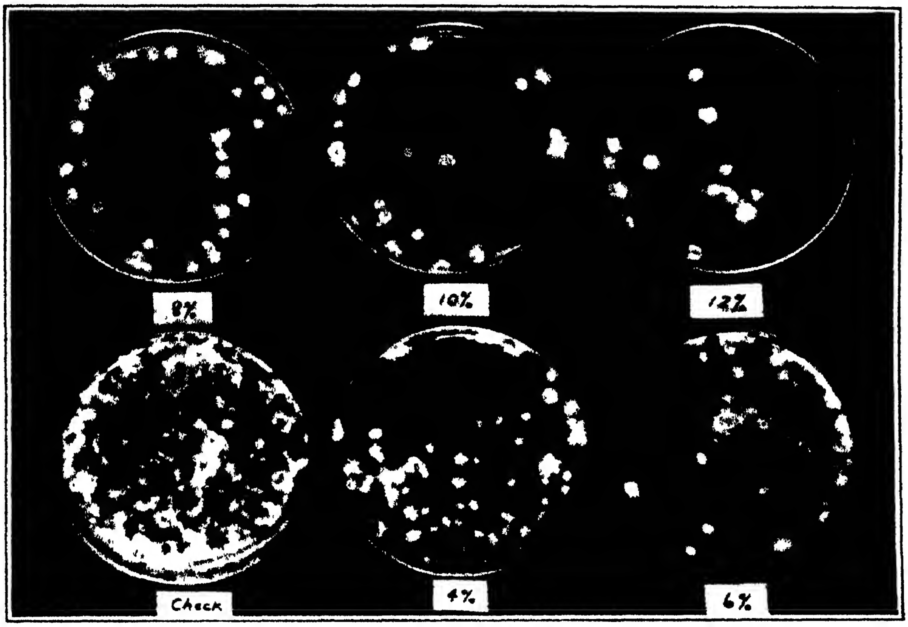


Fig. 4.—Effect of various concentrations of borax solution, at 110° F for 5 minutes, on spores of *Penicillium italicum*. The plates represent a 1:100,000 dilution of spore suspensions. The untreated suspension yielded numerous colonies, but treatments at 10 or 12 per cent killed large numbers of spores.

an intrinsic fungicidal action of the borax. Furthermore, in the case of *Penicillium digitatum* spores treated at 80° F, it seems that both germination and growth in plates are greater than at room temperature. This was true in Winston's (*41*) recent experiments. His conclusion is that increasing the temperature of borax was not ordinarily accompanied by a corresponding reduction in decay, except at 110° F. The comparative results are shown in figure 3.

Concentration.—Tests were made to determine the best concentration of borax solution. The concentration of the chemical ranged from 4 to 12 per cent in steps of 2 per cent, and the treatments were made at 110° F with a 5-minute period of exposure. The results are given in table 2, entries 65 to 76, and illustrated in figure 4.

The results show that as the concentration of borax solution increases the percentage viability of the treated spores decreases. Working on the control of decay in citrus fruit Barger (*4*) obtained a similar result. Winston (*41*) found that increasing the concentration of the borax solution up to 10 per cent, progressively decreased decay in the treated oranges; above 10 per cent, however, there was no marked increase in effectiveness of decay control.

INFLUENCE OF TEMPERATURES ON EFFECTIVENESS OF A MIXTURE OF BORAX AND BORIC ACID

From the result of experiments, Powell (*28*) suggested that the use of 2.5 to 5.0 per cent hot solutions of borax or boric acid, or a mixture of both gave complete control of green mold. This has been confirmed by Winston (*41*). He claimed, however, that boric acid, alone or in combination with borax, injured the rinds of oranges, grapefruit, and tangerines, although the effectiveness in decay control equaled that of borax.

Since borax and boric acid (2:1) solution is still commonly used in some packing-houses of California as an aid in washing as well as for better decay control, 8 tests including 2 checks were conducted at one time. For these treatments, the 6 per cent combined solution was adjusted to 100°, 110°, and 120° F and used for 5 minutes. The results are summarized in table 2, entries 77 to 84.

These results show that there is no appreciable reduction of germination at 100° F. A comparison with table 2, entries 27 to 34, shows, however, that the chemical solution is more effective than water at all three temperatures. As indicated by germination after 48 hours, the solution is more toxic to *Penicillium digitatum* than to *P. italicum*. At 110° F the combined chemical solution of borax-boric acid is slightly more toxic than is a 6 per cent borax solution; at 120° F the former is more toxic to *P. digitatum* but slightly less toxic to *P. italicum* than is the latter.

EFFECT OF METBOR

Metbor is a new material which has been mentioned by Stewart (*36*) as fully equal to borax in fungicidal efficiency, and as having very marked advantages over borax in regard to solubility in cold water and other properties. It is completely and quickly soluble in cold water in concentrations even greater than are necessary to obtain the equivalent of 8 per cent borax solution. In order to determine its effectiveness on spores of *Penicillium italicum* and *P. digitatum* as compared with borax, three separate experiments were conducted.

Time of Exposure.—This experiment consisted of 8 tests in which was used a 6 per cent solution of the chemical at room temperature (66° to 72° F) for 2, 5, 10, and 15 minutes respectively. The results are given in table 2, entries 85 to 94.

It is seen from the results that at the longest exposure time the germination after 24 hours is 65.8 per cent for *Penicillium italicum* and 49.6 per cent for *P. digitatum.* This shows much less reduction in germination than does borax under similar exposures.

Since the longest treatment was not effective, and since such treatments are impractical in the packing-house, 6 per cent Metbor has no usefulness at room temperature.

Temperature Relations.—Tests were made to determine temperature relations. Spores of both fungi were given 5-minute exposures to 6 per cent Metbor solution at temperatures of 66° to 72° F, 100°, 110°, and 120° F. The results are given in table 2, entries 95 to 104, and shown in figure 3.

In comparison with the results of the effect of temperature of distilled water (table 2, entries 27 to 34), 6 per cent Metbor is much more effective from the standpoint of spore germination, though the reduction in plate colonies is less significant.

Concentration.—Ten tests were made in which concentrations ranging from 4 to 12 per cent were used for 5 minutes at room temperature (66° to 72° F). The results are summarized in table 2, entries 105 to 116.

Although there is some reduction of germination and number of colonies at higher concentrations at 5-minute exposure, the results indicate that the chemical is not so effective in killing spores as borax.

EFFECT OF DINITRO-O-CYCLOHEXYLPHENOL

Since dinitro-o-cyclohexylphenol, used as a 0.01 per cent emulsion, has been shown to be effective in decreasing the number of brown-rot infections on lemon, caused by *Phytophthora citrophthora,* from 51.45 to 1.45 infections per fruit,[6] tests for its effectiveness on *Penicillium italicum* and *P. digitatum* were conducted, using three concentrations. The spores of the two fungi were treated separately at room temperature for 2 minutes. Results are tabulated in table 2, entries 117 to 124.

From the results it is evident that both spore germination and the growth on plates are but slightly affected by this substance under the conditions of the experiment although some reduction in number of colonies is noted as compared to the checks.

[6] Klotz, L. J., and L. L. Huillier. Dinitro-o-cyclohexylphenol as a treatment for brown rot of citrus. Unpublished data on file at Citrus Experiment Station, Riverside, California. 1936.

EFFECT OF SODIUM HYPOCHLORITE

Sodium hypochlorite (NaOCl) has been reported (*2, 3*) to be effective in controlling blue mold on apple and pear and in sterilizing packing rooms, etc. Klotz and Huillier[7] likewise found a 0.4 per cent solution completely effective in controlling brown rot of lemon.

To determine the toxicity of the chemical to blue and green molds of citrus, 6 tests were made with 0.4, 0.6, and 1.0 per cent solutions at room temperature for 2 minutes at each time; two checks were left without chemical treatment. The solutions were prepared by using a concentrated stock solution containing 6 per cent NaOCl. To prepare a solution containing 1.0 per cent available chlorine, the amount to be prepared is multiplied by 0.2. This gave the volume of stock solution to be used. The volume was made up with distilled water. The factor for 0.6 per cent is 0.111 and that for 0.4 per cent is 0.072.

The effectiveness of sodium hypochlorite is shown in table 2, entries 125 to 132. A low concentration (0.4 per cent) of the chemical in contact with the fungi for 2 minutes was completely lethal. Lack of success in some of the earlier experiments with this material was found to be due to an insufficient mixing and wetting of the mold spores.

Practically the hypochlorite has some disadvantages. Chlorine escapes, making it necessary to test and correct the treating solution frequently to maintain an effective concentration. The rate of loss of chlorine increases as the temperature of the treating solution is raised. This material is injurious to metal, cement, and wooden tanks. In the apple industry (*3*) these objections were in large measure overcome by use of a small, separate tank where the fruit was treated cold with hypochlorite solution stabilized by certain organic chemicals, and by maintaining the chlorine concentration by frequent colorimetric measurements with orthotolidine and the addition of concentrated sodium hypochlorite.

EFFECT OF SODIUM BICARBONATE

From the results of experiments in California, Barger (*5*) suggests the use of 3 to 5 per cent cold or hot solution of sodium bicarbonate for reducing mold. Negative results were obtained by Reichert and Littauer (*33*), who state that 3 and 5 per cent solutions of sodium bicarbonate for 5 and 15 minutes gave no control of wastage. Other results, however, have shown that 3 per cent and 5 per cent solutions of the chemical at 32° C

[7] Klotz, L. J., and L. L. Huillier. Sodium hypochlorite as a treatment for brown rot of citrus. Unpublished data on file at Citrus Experiment Station, Riverside, California. 1936.

(89.5° F) for 5 minutes gave some reduction of rot. Putterill and Davies (*31*) mentioned the beneficial use of 3 per cent sodium bicarbonate solution for controlling green mold. Recently Putterill (*30*) showed that 4 per cent sodium bicarbonate at high temperature was effective in controlling mold.

To determine the effect of sodium bicarbonate on spores of *Penicillium italicum* and *P. digitatum* for various periods of exposure and at several temperatures and concentrations of the chemical ($NaHCO_3$), 3 separate experiments were conducted. The effectiveness of sodium bicarbonate was also compared with that of sodium carbonate at several temperatures.

Time of Exposure.—Six tests were made with 6 per cent sodium bicarbonate at 86° F (30° C) for 2, 5, and 10 minutes respectively. The results are given in table 2, entries 133 to 140.

As indicated, a 6 per cent solution of sodium bicarbonate at 86° F and for a period of 10 minutes is practically inocuous to the spores of either mold. The plate tests show that the chemical slightly inhibited growth. These results are in contrast with those of Marloth (*22*), who found that a 6 per cent solution for 2 or 5 minutes was decidedly toxic to the spores of *P. digitatum*.

Temperature Relations.—With the aim of comparing more critically 6 per cent sodium bicarbonate solution with the same concentration of sodium carbonate (Na_2CO_3) these two substances were tested at the same time. The results are given in table 2, entries 141 to 158.

The lack of efficacy of sodium bicarbonate may be summed up by saying that the chemical at 120° F showed no advantage over water at that temperature. In fact the fungi after the bicarbonate treatment showed slightly greater viability than after the treatment in distilled water. Sodium carbonate, on the other hand, showed complete effectiveness at 120° F; and at 110° and 100° greatly reduced germination and growth.

It was also found that the temperatures of 6 per cent sodium bicarbonate solution below 120 F do not affect the viability of spores very much. This supports Barger's (*5*) conclusion that a treating temperature of 60° is as ineffective as one of 95° for this substance.

Concentration.—The effect of various concentrations of sodium bicarbonate solution was determined; the results are given in table 2, entries 159 to 166. The reduction in germination and growth of the two fungi was slight. With *Penicillium digitatum*, however, there is some reduction at 10 per cent but these tests show far less effect than is shown by the experiments of Marloth (*22*).

The results obtained by Barger (*4*) with experimentally injured and inoculated fruit show that 3 per cent sodium bicarbonate at 100° F re-

duces decay to 35.3 per cent and that 5 per cent sodium bicarbonate reduces it to 32.0 per cent. He concludes that a 3 per cent solution of sodium bicarbonate appears to be as effective in reducing mold on fruit as a 5 per cent solution.

EFFECT OF SODIUM CARBONATE

Sodium carbonate (Na_2CO_3) is used for controlling molds in many lemon packing-houses and in some orange houses in California. Doidge (*12*) has suggested the use of a 5 per cent solution of sodium carbonate for the control of *Penicillium* molds. As a result of comparative treatments of spores, Marloth (*22*) has concluded that the same concentration of carbonate is considerably more toxic than a similar concentration of bicarbonate. Recently Winston (*41*) has shown that a 3.5 per cent solution of sodium carbonate ($Na_2CO_3 \cdot 10H_2O$) at 80° F gave nearly the same result as that of the check without chemical treatment.

With the hope of obtaining more information in regard to the effectiveness of sodium carbonate in relation to time of treatment, temperature, and concentration, 3 separate experiments were conducted.

Time of Exposure.—Six tests with 6 per cent solution of sodium carbonate at 86° F were made, the spores being exposed for 2, 5, and 10 minutes.

The results shown in table 2, entries 167 to 174, indicate that the reduction of spore germination and growth in plates of both fungi become more significant as the time of exposure is extended. The results are more or less in harmony with those of Marloth (*22*).

Temperature Relations.—In this experiment, to determine temperature relations, the spores were treated with 6 per cent sodium carbonate at temperatures of 86°, 100°, 110°, and 120° F for 5 minutes. The comparative results are summarized in table 2, entries 144 to 149, and 154 to 158.

According to the results, it is shown that 6 per cent sodium carbonate at 120° F gave complete killing of spores of both fungi; that is, this treatment permitted neither germination nor the growth of a single colony.

In comparison with the results of the effect of temperature in distilled water, 6 per cent sodium carbonate at 120° F is much more effective in preventing germination than distilled water at the same temperature. This also shows that sodium carbonate apparently has an intrinsic fungicidal action on the two fungi not accounted for by the temperature of the treating solution.

It was also found that 6 per cent sodium carbonate solution at 110° F

was very toxic to both kinds of spores but this toxicity was slightly less than that at 120°.

Concentration.—In order to determine the best concentration of sodium carbonate for controlling blue and green molds, spores were treated with concentrations of 2, 6, and 10 per cent for 5 minutes at 86° F. The results are recorded in table 2, entries 175 to 182. They show that after 5-minute exposures the percentage of germination and the growth in plates of both fungi reduce significantly as the concentration increases. Under the conditions of this experiment, a 10 per cent solution of sodium carbonate was the most efficient concentration.

The results agreed with Marloth's (*22*) more in the case of *Penicillium italicum* than in that of *P. digitatum.*

A comparison of the results with those of sodium bicarbonate indicates that the same concentration of sodium carbonate was much more toxic than in the case of sodium bicarbonate.

RELATION OF TEMPERATURES TO EFFECTIVENESS OF CHLORAMINE-T

Klotz and Huillier[8] have shown that 0.4 per cent solution of chloramine-T reduces brown rot of inoculated lemons from 28.65 to 0.05 infections per lemon. To test the effect of this chemical in controlling blue and green molds, 0.4 per cent solution was used at room temperature, and at 100°, 110°, and 120° F, for 5 minutes. The effects of the various temperatures on the toxicity are given in table 2, entries 183 to 192. The reduction of spore germination and growth in plates of both fungi is very marked at higher temperatures, especially at 120° F. No germination and growth of either fungus were found at 120°. Chloramine-T is apparently more toxic to *Penicillium italicum* than to *P. digitatum* at the three higher temperatures used. At temperatures used below 110°, it showed but slight toxicity.

EFFECT OF OTHER SUBSTANCES

Sodium o-phenylphenate at 0.15 per cent has been used by Klotz and Huillier[9] in unreported experiments on the control of brown rot of lemons. They show that a 0.15 per cent solution reduces the rot of inoculated fruit from 28.65 to 0.55 infections per lemon. A commercial

[8] Klotz, L. J., and L. L. Huillier. Chloramine-T as a treatment for brown rot of citrus. Unpublished data on file at the Citrus Experiment Station, Riverside, California. 1936.

[9] Klotz, L. J., and L. L. Huillier. Sodium o-phenylphenate as a treatment for brown rot of citrus. Unpublished data on file at the Citrus Experiment Station, Riverside, California. 1936.

washing powder containing mostly soda ash with some caustic and a trace of pine oil, has also been commonly used in some of the packing-houses in California, and chloramine-T is reported to have been tried.

An experiment was set up to test the relative effectiveness of these three substances. The time of exposure was 5 minutes, the temperature, 66° to 72° F, and the concentrations as follows: sodium o-phenylphenate, 0.15 per cent; chloramine-T, 0.4 per cent; and washing powder, 1.0 per cent. The results are shown in table 2, entries 193 to 200.

Under the conditions used, sodium o-phenylphenate is the most effective substance of the three in reducing germination and the growth of *Penicillium digitatum;* it was also slightly more effective than chloramine-T and washing powder in reducing germination of *P. italicum.* The latter two substances are almost without effect on *P. digitatum* under the conditions mentioned above. With *P. italicum,* 0.4 per cent chloramine-T is more effective in reducing germination and the number of colonies than is 0.15 per cent sodium o-phenylphenate or the 1 per cent washing powder.

DISCUSSION

The inhibiting or the lethal effect of a given solution on the spores of a fungus is dependent upon a number of factors, including concentration of the fungicide and the spore suspension, duration of exposure, solvent for the fungicide, temperature, H- and OH-ion concentration, and the characteristically specific nature of the cations and anions. In the tests reported in this paper at the lower temperatures of 69°, 86°, and 100° F, and with a 5-minute treatment (2 minutes for sodium hypochlorite) the solutions in order of toxicity from highest to lowest are as shown in table 3, the numbers in the fourth column being the relative viability of the treated spores based on the combined mean of the percentage of germination after 24 hours and the viability on a culture medium.

The sodium hypochlorite solutions acting for only 2 minutes were fatal to the spores of both *Penicillium italicum* and *P. digitatum.* These results are similar to those of Baker and Heald (*3*) who found that rinsing apples for one minute with a sodium hypochlorite solution containing 0.4 per cent available chlorine was very effective to reducing the number of viable spores of *P. expansum* on the surface and in the lenticels, and in decreasing losses from decay by this organism.

Sodium carbonate occupies a relatively high position in the toxicity tables, and shows a greater effect on *Penicillium italicum* than on *P. digitatum.*

Borax in the cool solutions was more toxic to *P. digitatum* than to *P.*

TABLE 3

AVERAGE VIABILITY OF THE SPORES AFTER TREATMENT WITH VARIOUS FUNGICIDES AT VARIOUS TEMPERATURES

Penicillium italicum				*Penicillium digitatum*			
Order of toxicity of fungicide	Fungicide	Concentration of fungicide, per cent	Viability*	Order of toxicity of fungicide	Fungicide	Concentration of fungicide, per cent	Viability*
Treatment at 69°, 86°, and 100° F							
1	Sodium hypochlorite†	1.0	0.0	1	Sodium hypochlorite†	1.0	0.0
2	Sodium hypochlorite†	0.6	0.0	2	Sodium hypochlorite†	0.6	0.0
3	Sodium hypochlorite†	0.4	0.0	3	Sodium hypochlorite†	0.4	0.0
4	Sodium carbonate	6.0	30.4	4	Sodium o-phenylphenate	0.15	7.3
5	Sodium o-phenylphenate	0.15	38.2	5	Castile soap for 10 minutes followed by borax	0.25 6.0	33.8
6	Castile soap for 10 minutes followed by borax	0.25 6.0	58.4	6	Sodium carbonate	6.0	48.3
7	Chloramine-T	0.4	58.0	7	Borax	6.0	56.5
8	Borax-boric acid (2:1)	6.0	58.15	8	Metbor	6.0	78.1
9	Borax	6.0	58.17	9	Sodium bicarbonate	6.0	87.7
10	Dinitro-o-cyclohexylphenol†	0.001	70.1	10	Dinitro-o-cyclohexylphenol†	0.001	88.2
11	Metbor	6.0	74.7	11	Washing powder	1.0	91.2
12	Washing powder	1.0	79.7	12	Borax-boric acid (2:1)	6.0	94.1
13	Sodium bicarbonate	6.0	83.9	13	Chloramine-T	0.4	93.5
Treatment at 110° and 120° F							
1	Chloramine-T	0.4	0.1	1	Sodium carbonate	6.0	0.6
2	Sodium carbonate	6.0	0.5	2	Chloramine-T	0.4	10.4
3	Borax-boric acid	6.0	4.6	3	Borax-boric acid	6.0	11.1
4	Borax	6.0	19.3	4	Metbor	6.0	20.6
5	Metbor	6.0	31.1	5	Borax	6.0	22.4
6	Sodium bicarbonate	6.0	34.8	6	Sodium bicarbonate	6.0	44.1

* Calculations all based on percentage germination after 24 hours and relative viability, being related to germination and relative viability of water check (100).

† All treatments for 5 minutes except in the case of dinitro-o-cyclohexylphenol and sodium hypochlorite treatments of 2 minutes.

italicum, although the borax-boric acid mixture had a greater effect on the latter.

Sodium o-phenylphenate was effective on both fungi but more toxic to *Penicillium digitatum.*

Cold 6 per cent solutions of Metbor and of sodium bicarbonate were relatively ineffective on the two fungi. A 0.4 per cent solution of chloramine-T decreased the viability of *P. italicum* 42 per cent but *P. digitatum* only 7 per cent.

At the higher temperatures of 110° and 120° F, and with a 5-minute treatment (6 minutes at 110° F in one experiment with borax), the order of toxicity of the several solutions is shown in table 3.

One of the striking features of this record is the toxicity positions occupied by the warm chloramine-T and borax-boric acid solutions as compared with those of the solutions at the lower temperatures. Another surprising result is the consistently greater resistance of *Penicillium digitatum* to the growth inhibition of the several solutions. This would indicate that *P. digitatum* would be found more difficult to control with the solutions mentioned above than would *P. italicum.* But most of the results of experiments on decay prevention in citrus fruits show that borax or borax-boric acid is much more effective against the green than against the blue mold. As one possible explanation for this difference may be offered the suggestion that when the solution comes in contact with the oil of the rind, some chemical action takes place whereby the original specific toxicity of the treating solution to the particular spores is altered. Another explanation may be that some of the treating substances remaining on the rind surface or in injuries may react differently to germinated spores of one fungus than to those of the other. Marloth (*22*) found that germinated spores of both fungi were more readily killed by borax, sodium carbonate, and sodium bicarbonate than nongerminated spores.

The comparative results are shown in figure 3. Sodium bicarbonate again shows the least toxicity, while sodium carbonate averages higher for the two fungi. The importance of temperature is readily seen from the above and from a consideration of the results in table 2. The lower toxicity at the lower temperatures may be due in part to poor wetting of the spores, although that factor was decreased as much as possible by the pretreatment with soap solution. The greater toxicity of the chemical solutions at the higher temperatures should be largely related to the increased velocity and penetrating power of the toxic ions and molecules and to the kinetic energy of the particles of the solvent itself, since water at 120° F greatly inhibited subsequent germination. A 5-minute exposure

to sodium carbonate, borax-boric acid mixture, chloramine-T, and Metbor at 120° F was fatal to the spores of the two fungi, while water, sodium bicarbonate, and borax at the same temperature and exposure permitted survival of a small percentage. At 110° F nearly all the spores of *Penicillium italicum* were killed by chloramine-T and the borax-boric acid mixture. Not all the spores of *P. digitatum* were killed by any of the solutions tried at 110° F, although 6 per cent sodium carbonate permitted only 0.4 per cent germination after 24 hours and a relative viability of only 1.9.

As would be expected, the longer the period of exposure to any of the several chemicals used, the smaller the percentage of germination of either fungus; this is especially striking in the experiments with 6 per cent borax solution at 110° F. The comparative results are given in figure 5.

The results of the concentration experiments show that all the spores of both blue and green molds were killed at room temperature by the weakest solution (0.4 per cent) of sodium hypochlorite tried. After treatment with 12 per cent borax solution at 110° F, no spore germinations were observed after 24 hours. Conidia of *Penicillium italicum* treated with 8 per cent borax solution at 110° F were affected significantly, while those of *P. digitatum* were affected significantly in 10 per cent borax solution at the same temperature. A 10 per cent solution of Metbor at 66° to 72° began to reduce the germination of *P. italicum* markedly. In the case of *P. digitatum* spores were affected significantly in 12 per cent Metbor solution at 66° to 72°. From this point of view, it is also indicated that *P. digitatum* is more resistant to the toxicity of higher concentrations of borax and Metbor than *P. italicum*. Both spores began to be affected by a 10 per cent sodium carbonate solution at 86° F. Spores of *P. digitatum* and *P. italicum* were but slightly affected by a 10 per cent solution of sodium bicarbonate at 86° F, although the former were less inhibited than the latter. The differences among concentrations of dinitro-o-cyclohexylphenol as to their effect upon spores of both fungi were not sufficiently great to be of practical significance.

As far as the results of these experiments are concerned, it may be concluded that the most effective and economical solution for killing blue and green mold spores is the 0.4 per cent sodium hypochlorite used for 2 minutes at room temperature. The following solutions may also be effective when used at 120° F for 5 minutes: 0.4 per cent chloramine-T, 6 per cent mixture of borax and boric acid, 6 per cent sodium carbonate, and 6 per cent Metbor. Eight to 12 per cent solutions of borax are effective if used at 110° for 14 to 16 minutes.

As Fawcett (*14*) has pointed out, 120° F for 2 to 4 minutes is gener-

ally considered the danger point for the temperature of washing or treating solutions. With freshly picked fruit, especially with lemons, it is

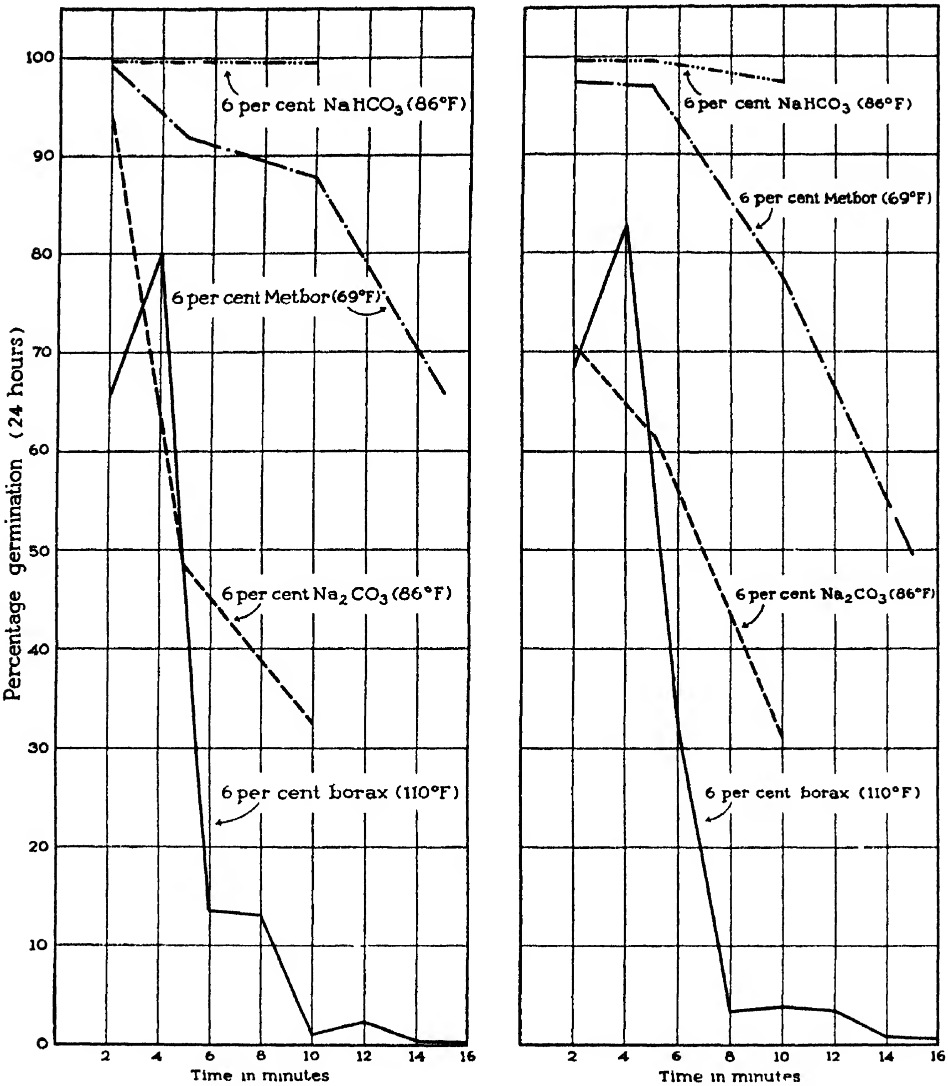


Fig. 5.—Left: Comparative effect on spore germination of *Penicillium italicum* of various exposures to four different solutions at recommended temperatures. Six per cent borax solution at 110° F for 10 minutes or longer killed nearly all the spores.
Right: Effect of the same solutions on the spore germination of *Penicillium digitatum*. Six per cent borax solution at 110° F for 8 minutes or longer killed nearly all the spores.

necessary to use lower temperatures or to dry the fruit for several days before treatment, as pointed out by Fawcett and Klotz (*16*). To avoid injury to freshly picked fruit and at the same time secure partial control

of blue and green molds the four solutions mentioned above may be used at 110° to 115° F, providing the fruit is first allowed to dry 3 to 5 days.

The spores showed a great tolerance toward hydroxyl ions. A 5-minute treatment in 2 per cent Castile soap having a pH 10.05 had no effect on the subsequent viability of the spores. A 2-minute treatment in 6 per cent soda ash solution (pH 10.16) and a 5-minute immersion in a solution of 1 per cent washing powder (pH 9.90) had but slight effect on germination. However, a 2-minute exposure to the 0.4 per cent alkaline sodium hypochlorite solution (pH 11.1) was fatal. While the high OH-ion concentration was likely an important factor in the toxicity of the hypochlorite solution, the lethal effects may have been due in a large measure to the toxicity of free chlorine, OCl ions, and nascent oxygen. Klotz (*21*) has shown that clorine even in small concentration is lethal to *Penicillium italicum* and *P. digitatum*. Marloth (*22*) found that the spores of the two fungi showed abundant germination in a slightly buffered Duggar's solution to which orange extract had been added and which had been adjusted to the acidity-alkalinity range of pH 2.4 to pH 9.4. In the range pH 3.0 to pH 9.2 the germination in that medium was relatively indifferent to H-ion concentration. No germination of either organism was obtained in Sörensen's glycocoll buffer above pH 9.7 or in 2.6 and 10.0 per cent solutions of sodium carbonate, potassium carbonate, sodium bicarbonate, and potassium bicarbonate, or in 4 per cent sodium tetraborate solution. Since the estimations of H-ion concentration in those studies were made with the quinhydrone electrode they are reliable only up to approximately pH 7.5. For example, the pH of 10 per cent solutions of bicarbonate and carbonate which were reported as 8.6 and 11.4, respectively, were estimated by the glass electrode and reported in the present paper as pH 8.0 and pH 9.9.

The results seem to indicate that $\overset{+}{\mathrm{H}}$ and $\overline{\mathrm{OH}}$ ions in the concentration range of pH 2.4 to pH 10.0 are in themselves relatively innocuous to the spores of *P. italicum* and *P. digitatum*. However, owing to their possible effect in altering the permeability of the fungus protoplasts these very mobile ions may affect the results with other toxic ions. Osterhout (*25, 26*) found that alkali increases permeability, and that acid at first decreases then rapidly increases permeability in the seaweed *Laminaria saccharinum*.

Unlike the technique of the former paper (*22*) in which the spores were germinated directly in media whose pH was adjusted, the procedure of the present paper exposed the spores for only a short period to the action of the $\overline{\mathrm{OH}}$ and other ions, then rinsed with water and mounted them in a medium favorable for germination. The short period of exposure to

the toxic solution followed by a rinse would correspond to that treatment usually given in a packing-house. Some packing-house procedures, however, as in the case of the water-wax method for lemons, allow the toxic solution, containing soda ash in this case, to dry on the fruit and thus maintain a protective coating. As suggested by Marloth (*22*) the toxic salt thus deposited would form a relatively concentrated solution in condensation water that might subsequently form on the surface of the fruit and would, by killing the tender swollen spores and germ tubes, repulse invasion of the fungi that might lodge in that water.

Some discrepancies are seen between the results of the germination tests and those of the dilution plate methods. These may be due to the clumps of spores forming individual colonies which would be recorded as arising from single spores.

SUMMARY

To obtain information on the toxicity of various chemical solutions, at several temperatures and concentrations, to *Penicillium italicum* and *P. digitatum* (the causal agents of blue and green mold of citrus fruits), the spores of the fungi were immersed for certain time periods, and their subsequent viability compared with that of untreated spores by means of germination and dilution-plate tests. The technique of the methods employed is described in detail.

It was shown that a 0.25 per cent solution of a nontoxic soap effectively wets and prepares the spores of *Penicillium italicum* and *P. digitatum* for the chemical treatment that follows. No decrease in germination followed the pretreatment with the soap.

Distilled water at 120° F for 5 minutes killed approximately 90 per cent of the spores.

Tests in which 6 per cent borax at 110° F was used for 2, 4, 6, 8, 10, 12, 14, and 16 minutes, and at room temperature (66° to 72°), 80°, 100°, 110°, and 120° F for 5 minutes, and at concentrations of 4, 6, 8, 10, and 12 per cent for 5 minutes at 110° F, showed, as would be expected, that the longer the exposure to, the higher the temperature of, and the greater the concentration of the chemical, the more effective was the solution in reducing viability. Similar relations were found with sodium carbonate and Metbor.

Under the conditions of the experiments toxicity of the several solutions to spores of *Penicillium italicum* and *P. digitatum* was more dependent on temperature than on concentration of the chemicals or the period of immersion. A 5-minute exposure at a temperature of 120° F

in a 6 per cent borax-boric acid mixture, or 6 per cent Metbor, or 0.4 per cent chloramine-T, or in 6 per cent sodium carbonate, was lethal to the spores of both fungi. Details of the effects of the several temperatures may be secured from tables 2 and 3.

A saturated solution of dinitro-o-cyclohexylphenol and a 1 per cent proprietary washing powder used at room temperature for 2 minutes and 5 minutes, respectively, showed only a slight inhibitory effect on spore germination.

A 5-minute exposure of the spores in 6 per cent sodium bicarbonate at 86°, 100°, 110°, and 120° F showed no advantage of the chemical over water. At 86° F, immersion in a 10 per cent solution of sodium bicarbonate for 5 minutes or in one of 6 per cent for 10 minutes, had but little effect on the spores.

Two-minute exposures to 0.4, 0.6, and 1.0 per cent solutions of sodium hypochlorite were fatal to the spores of both fungi.

Excluding the sodium hypochlorite solutions which killed all the spores of both fungi, the three most efficacious solutions, when used at 100° F and below for 5 minutes, were 6 per cent sodium carbonate, 0.15 per cent sodium o-phenylphenate and 6 per cent borax; at 110° and 120° F the 3 most toxic were 0.4 per cent chloramine-T, 6.0 per cent sodium carbonate, and the 6 per cent mixture (2:1) of borax-boric acid.

ACKNOWLEDGMENTS

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LEAF-SCAR INFECTION IN RELATION TO THE OLIVE-KNOT DISEASE

WM. B. HEWITT

LEAF-SCAR INFECTION IN RELATION TO THE OLIVE-KNOT DISEASE[1,2]

WM. B. HEWITT[3]

INTRODUCTION

OLIVE KNOT, a serious disease of *Oleo europea* L. in most of the olive-producing districts north of the Tehachapi mountains of California, is characterized by the development of overgrowths, knots on the branches. These knots develop most frequently at the leaf scars except in years when freezing injury occurs. The present study furnishes inoculation data, with histological evidence that the scars, under certain conditions, are portals of entry of the causal agent, *Bacterium savastanoi* E.F.S., into the host. This paper also deals with inoculation experiments used to determine the length of time during which the leaf scars are susceptible to infection. It further describes the microchemical and histological studies of the development of the abscission region just before and after leaf fall, and thereby elucidates the rôle played by leaf scars in infection.

PREVALENCE OF LEAF-SCAR INFECTION

Observations in California olive groves show that a large percentage of the new knots forming each year develop at leaf scars. Horne, Parker, and Daines (*2*)[4] were first to point out this fact: "By far the largest number of knots appear on leaf scars or wound callus." Wilson (*6*) also recognizes the importance of leaf scars as infection courts. He points out that in years other than those in which freezing injury occurs, as high as 90 per cent of the new knots on branches develop at leaf scars.

The distribution of inoculum from active knots necessary to infect leaf scars depends upon the presence of dripping moisture, as from rain. Horne, Parker, and Daines (*2*) state that the bacteria exude in a slimy mass from the fissures of knots during rain. Wilson (*6*), in further and more extensive studies, demonstrates the importance of rain in the exudation and spread of the organism as related to infection. He shows that enough bacteria were present to cause infection within a short period after the knots were moistened. He placed wounded, healthy, potted trees under diseased trees, allowed a fine spray of water to fall over them,

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[2] A thesis submitted in partial fulfillment of requirements for the degree of Doctor of Philosophy, University of California, 1936.

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[4] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

and then placed the potted trees in the greenhouse so that the knots might develop. In this experiment he reports numerous knots developing on trees removed from the spray 7 minutes after it was started. Wilson also infected newly formed leaf scars by artificial inoculation and showed

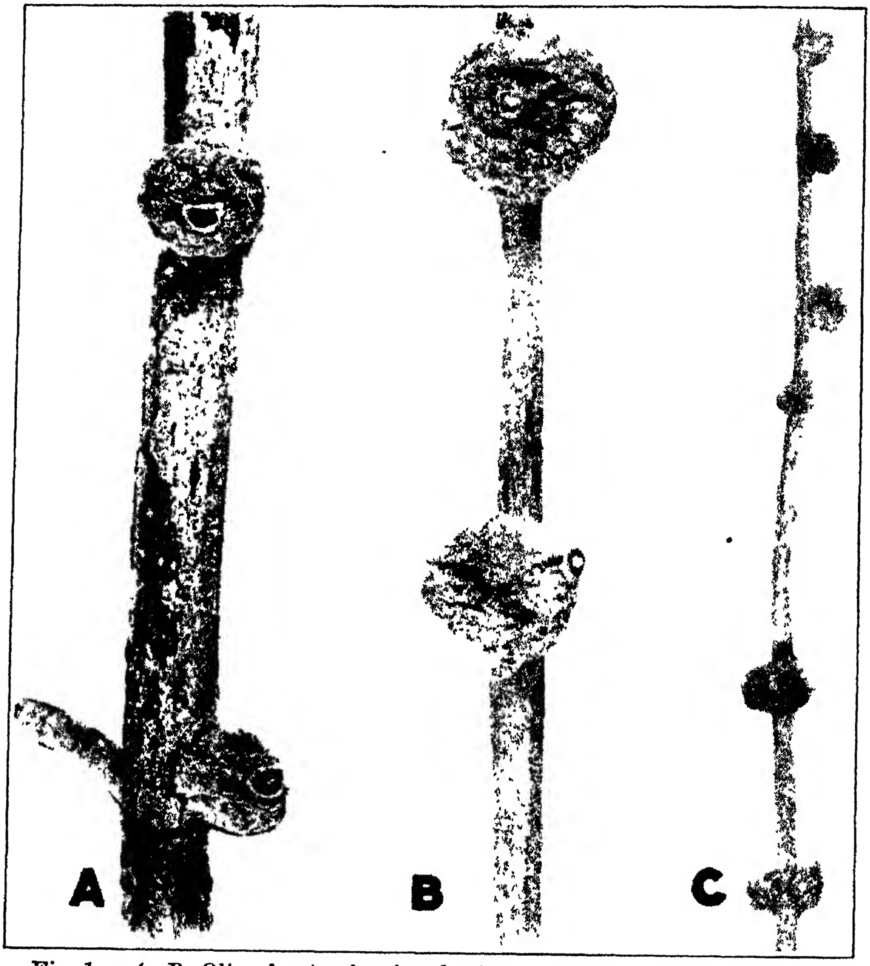

Fig. 1.—*A*, *B*, Olive knots showing leaf scars on the surface. *C*, Young olive knots resulting from natural leaf-scar infections. (*A*, ×2; *B*, ×4; *C*, natural size.)

that the vessels in the leaf scars were open at the time of leaf fall. According to his climatic studies, infection may occur in a range of temperature from about 40° to 100° F.

The origin of the knots can be determined by examination. Those developing at the nodes usually form at the leaf scar, which upon close inspection, can usually be seen on the surface of young knots (fig. 1).

To gain further evidence as to the importance of leaf-scar infection, the following experiments were carried out. On May 5, 1936, in a Mission-olive orchard in Sacramento County, 60 healthy branches were tagged. When these were re-examined in October, 241 knots were discovered, of which 225 (about 93.5 per cent) were located at leaf scars.

On May 14, 1936, in the same olive orchard 339 yellow leaves about ready to fall were removed by bending them slightly backward. This leaf removal was continued until interrupted by a shower. Other light showers occurred on May 28. By August, 229 of the leaf scars (67.5 per cent) had developed knots.

The olive normally drops some of its older leaves each season, and the time of abscission of leaves plays an important part in the infection of leaf scars. Wilson (*6*), studying natural leaf fall, finds that the period of maximum fall varies with the season; and, in unpublished experiments, shows that leaf drop generally starts about the time growth begins in late January or February, rises slowly during the early months, and increases suddenly to a maximum at full bloom around the middle of May. It then decreases through the summer and usually ceases by the latter part of September. Observations by the writer during the seasons of 1935 and 1936 confirmed those of Wilson.

MATERIALS AND METHODS

Choosing Material.—All experiments, unless otherwise stated, were conducted on the Mission variety of olive in an orchard in Sacramento County, California.

Fresh leaf scars were made by removing only the leaves that were three-fourths to entirely yellow. In such leaves the abscission process was well advanced, and the leaves were about to drop. The leaves were removed by placing a pencil or finger at the tip of the leaf and bending them toward the base of the stem. If the leaf fell by the time it was pushed back one half of the distance to the main limb—that is, approximately 45°—the resulting scar was encircled with a red wax pencil mark and retained for use in these studies.

Method of Inoculation.—In artificial inoculations a water suspension of *Bacterium savastanoi* E.F.S. from a 36- to 48-hour culture on potato dextrose agar was used. A drop of this suspension was placed over the leaf scar with a small camel's hair brush.

Collecting Leaf Scars.—Leaf scars were collected by cutting out a small portion of the stem along with the leaf scar (fig. 2, *B*).

Moist Chambers.—Moist conditions were provided by enclosing a

branch in a cylinder of wire gauze (⅛-inch mesh screen) and afterwards wrapping it with three layers of wet cheesecloth. The bottom of the chamber was then placed in a can containing water, and the can was suspended by a wire from a large branch in the tree (fig. 2, *A*).

Method of Holding Fresh or Killed Unimbedded Leaf Scars for Sectioning.—Sliding microtome sections of leaf scars killed, fixed, and stored in formalin-acetic-alcohol solution, together with sections of fresh

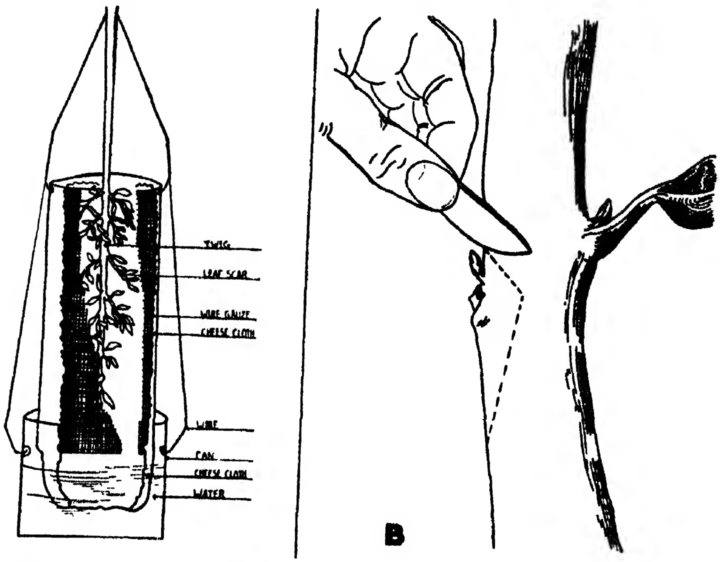

Fig. 2.—*A*, Diagram of the moist chamber used. *B*, Illustrating the method used for cutting leaf scars from the stems before fixation.

leaf scars, were used for these studies. The collected scars were small and in order that they might be sectioned without imbedding, a method was devised to hold the scars. They were mounted directly in paraffin blocks. The tissue was first blotted until the surface appeared dry. A small hole was melted in one end of a paraffin block with a hot iron rod, and the scar was then inserted into the melted paraffin. To loosen air bubbles adhering to the scars, a warm dissecting needle was moved around the material; this also served to orient the scars in the blocks. The paraffin block was then cooled in ice water, trimmed, and sectioned by the sliding microtome. Most of the sections of the leaf scars freed themselves from the surrounding paraffin when placed in water, but sections that adhered to the paraffin were freed easily by prodding with a small brush.

Paraffin Method Used.—The material was killed and fixed in a solution of formalin 10 cc, acetic acid 10 cc, and 50 per cent alcohol 100 cc for 12 hours or longer. It was imbedded in paraffin as follows: (A) The speci-

mens were transferred directly from the formalin-acetic-alcohol into a solution of 10 per cent glycerin in water. The solution was then allowed to evaporate until it became thick. (B) The material was next successively transferred through the following glycerin and normal butyl alcohol mixtures and left in each for 48 to 72 hours:

(a) 75 parts of glycerin and 25 parts of normal butyl alcohol.
(b) 50 parts of glycerin and 50 parts of normal butyl alcohol.
(c) 25 parts of glycerin and 75 parts of normal butyl alcohol.
(d) Pure dehydrated normal butyl alcohol; four changes—the first two 24 hours apart, the next two 48 hours apart—were used. The containers were placed on top of the paraffin oven to warm; this hastened the removal of all the glycerin.

(C) Small amounts of paraffin were added to the last change of butyl alcohol while the specimens were still on top of the oven, and they were allowed to stand for 12 hours. Then the process of infiltration was completed as in standard schedule (*5*).

INOCULATION OF THE ABSCISSION ZONE BEFORE LEAF FALL

If infection of the leaf abscission zone were to precede leaf fall, it might be expected to occur through a break in the epidermis or some other open infection court. This point was tested by inoculating the base of the petiole with a water suspension of *Bacterium savastanoi*. On May 31, 1935, 50 yellow leaves were inoculated at the base of the petiole. When examined on August 31, only 2 out of the 50 leaf scars had developed knots. Again on May 4, 1936, the base of the petioles of 67 yellow leaves were inoculated. The number of leaves that had fallen at intervals after inoculation were as follows: at the end of 1 hour, 29; 3 hours, 42; 24 hours, 55; and 48 hours, 64. By September 1, 2 of the 67 leaf scars had developed knots. The leaves from the leaf scars that developed knots probably fell shortly after inoculation, which allowed the inoculum to spread over the scar and accomplish infection.

The condition of the epidermis of the petiole in the region of the abscission zone was studied in 25 yellow leaves. A break that might serve as an infection court was found at the abscission zone in the axil of only one leaf. Figure 3 shows a photograph of this break. Judging from these results and from the inoculations previously described, infection of leaf scars is not apt to occur before leaf fall.

THE NEWLY FORMED LEAF SCAR

A microchemical and histological study of the abscission zone of 25 yellow Mission-olive leaves was made before and after leaf fall to determine what changes take place in this zone, because the structure and composition of these tissues were suspected to have an important relation to the infection process.

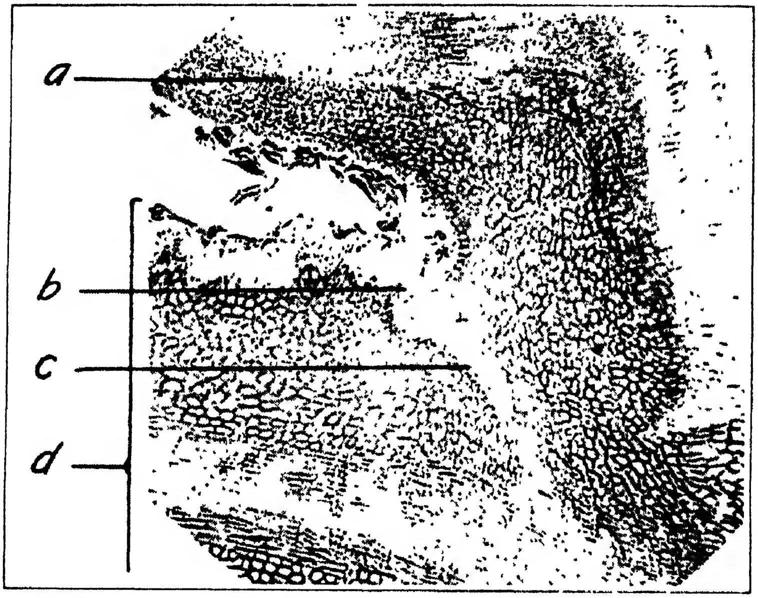

Fig. 3.—A portion of a longitudinal section through the abscission zone of a yellow leaf, showing a break in the epidermis in the axil of the leaf. Details are: *a*, Bud; *b*, a break in the epidermis at the edge of the abscission zone in the axil of the leaf; *c*, abscission zone; *d*, leaf petiole. (× 55.)

The mode of abscission in the olive leaf resembled that of *Castanea sativa* Mill., as described by Lee (*4*).

The abscission zone of the olive leaf lies at the base of the leaf petiole very close to the stem and, in longitudinal sections, stains lighter than the surrounding tissues (fig. 3). The number of layers of cells involved in the abscission zone varies from 1 to 7, but is more frequently 3 to 5. The zone extends from the epidermal cells through the entire diameter of the petiole and involves all the living cells. Leaf fall does not occur until the leaf is almost completely yellow. Separation takes place along an irregular plane between the walls of cells in the portion of the zone proximal to the stem.

In no leaves studied were there any cell divisions before separation,

nor any protective layers of lignin, gum, or suberin formed in the tissues above or below the abscission zone. The cuticle, sieve tubes, vessels, and fibers were mechanically broken at leaf fall. The epidermal cells at the separation plane of the scar appeared as though they had been broken at leaf fall. Just after leaf fall, therefore, the scar was an open wound, with exposed unprotected tissue, and open vessels.

SUSCEPTIBILITY OF NEWLY FORMED LEAF SCARS TO INFECTION

In June, 1935, leaf scars made by removing yellow leaves were inoculated immediately after their formation. Some were placed in the moist chambers for incubation; others were left exposed to outside atmospheric conditions. The inoculations were made when the outside air temperature was 28.5° C in the shade and when a gentle north wind was blowing. The temperature in the moist chambers ranged from 20° to 21° C. Of the 196 leaf scars inoculated and placed in moist chambers for 24 hours' incubation, 156 (or 79.6 per cent) developed knots; and of the 224 left outside of moist chambers 184 (or 82.6 per cent) developed knots. The data show that inoculations made immediately after leaf fall produced a high incidence of leaf-scar infection, and indicate that moist chamber conditions are not essential to a high degree of infection.

PERIOD DURING WHICH LEAF SCARS ARE SUSCEPTIBLE TO INFECTION

The period during which leaf scars are susceptible to infection was determined by periodic inoculations after scars were formed.

The leaf scars of one series were left outside of moist chambers until inoculation at which time they were placed in moist chambers for incubation and left 24 hours, after which the moist chambers were removed. The leaves for a second series were removed on the same dates, the scars being placed in moist chambers when formed, to determine the influence of high atmospheric humidity on scar healing and its relation to infection. After the moist chambers had been removed a short time for inoculation, they were replaced and left over the scars for 24 hours after inoculation, then removed.

Groups of leaf scars in each series were inoculated at intervals after they were formed. Series of inoculations were made during June, 1935; made again in May, 1936, because of an early season; and repeated at shorter intervals in June, 1937. The scars were allowed to remain on the

trees until knots were well developed (fig. 1). The results are given in table 1, and presented graphically in figure 4.

As shown in figure 4, the percentage of leaf scars susceptible to infection decreased rapidly within the first day. This drop is much more pro-

TABLE 1

RESULTS OF INOCULATIONS OF LEAF SCARS AT VARIOUS TIME INTERVALS AFTER LEAF REMOVAL

Age of leaf scar at time of inoculation (days)	Inoculations of 1935		Inoculations of 1936		Inoculations of 1937		
	Number of leaf scars inoculated	Per cent of leaf scars forming knots	Number of leaf scars inoculated	Per cent of leaf scars forming knots	Age of leaf scar at time of inoculation (hours)	Number of leaf scars inoculated	Per cent of leaf scars forming knots
Scars left outside moist chambers, except for 24-hour inoculation period							
0	11	81 8	46	97.9	0	51	96.5
1	14	57.2	37	64 8	1	36	100.0
2	12	41.6	45	57.8	3	24	99.2
3	12	25 6	38	39.5	6	24	79.2
4	10	40.0	33	42.4	12	22	68.2
5	12	33.0	44	34 1	24	23	52.2
7	12	8.3	37	13.5	48	23	42.4
8		.	40	15.0	72	35	31.7
9		.	14	7 0	96	45	28 9
10			12	0			
13	11	0					
15	12	0					
21	10	0					
Uninoculated	15	0	34	14 7	Uninoculated	25	0
Scars kept in moist chambers until 24 hours after inoculation							
0	20	85.0	80	90 0	0	51	96.5
1	24	29.2	74	24 3	1	25	92.0
2	30	3 3	64	17.2	3	20	100.0
3	39	5.1	39	12.9	6	20	85.0
4	16	6.2	98	9.2	12	20	75.0
5	10	0	60	1.7	24	22	18.2
6		..	25	0	48	22	13.6
7	16	0		.	72	24	8.0
11	11	0	.	.			
Uninoculated	10	0	51	.	Uninoculated	15	0

nounced in the scars kept in moist chambers than in the scars left outside. According to these data, leaf scars left outside of moist chambers are susceptible to infection longer than those kept in moist chambers. The latter became immune about the fifth day, whereas the former did not

become immune until they were 7 to 9 days old. The infection of some of the scars used as controls invalidates the curve of 1936 inoculations after the sixth day, since any infection shown after this time may have resulted from earlier natural infection that prevented the curve from dropping to zero. The United States Weather Bureau in Sacramento recorded a

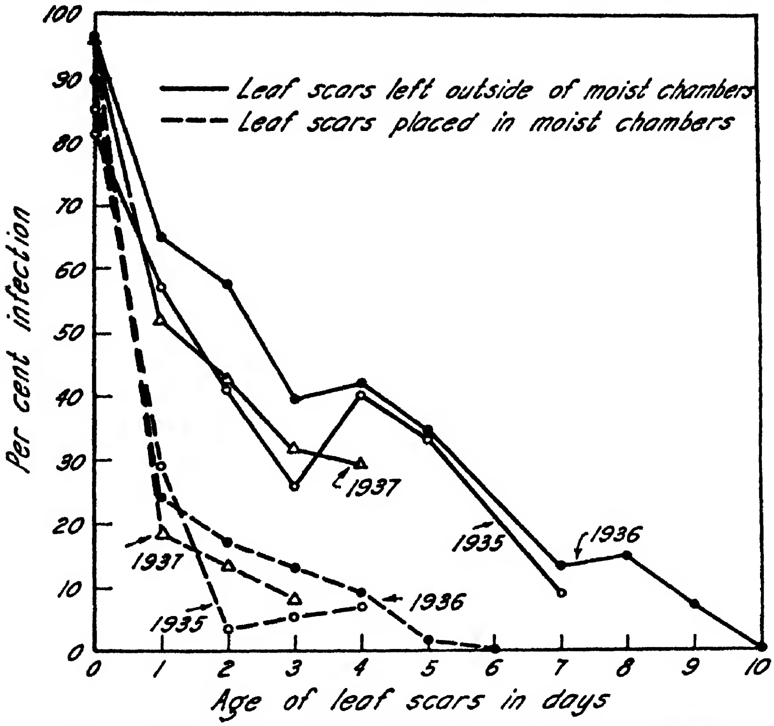

Fig. 4.—Showing the relation between the age of the leaf scars in days at the time of inoculation with *B. savastanoi* and the per cent of scars developing knots for both those kept in moist chambers and those left outside.

trace of rain on May 13 and 0.30 inch on May 14. These rains, occurring on the fifth and sixth days of the series of inoculations, might have initiated some infection in the experimental scars.

In 1937 the inoculations were made at shorter intervals to discover when the drop in infected scars takes place through the first 24 hours (table 1 and fig. 5). During the first 12 hours, the percentage of scars infected at each inoculation period remained about the same, both for those scars kept in moist chambers and for those left outside. In the former, the infected scars dropped from 75 per cent at the 12-hour period to only 18 per cent at the 24-hour period, while in the latter group the percentage of scars infected dropped gradually at each inoculation after 3 hours.

Though the number of scars used in any one year's inoculation is small, the results for the three years consistently show that leaf scars soon become immune to infection (fig. 4).

If the conditions within the moist chambers might simulate a rather long period of high humidity, such as might occur with a rain of a week's

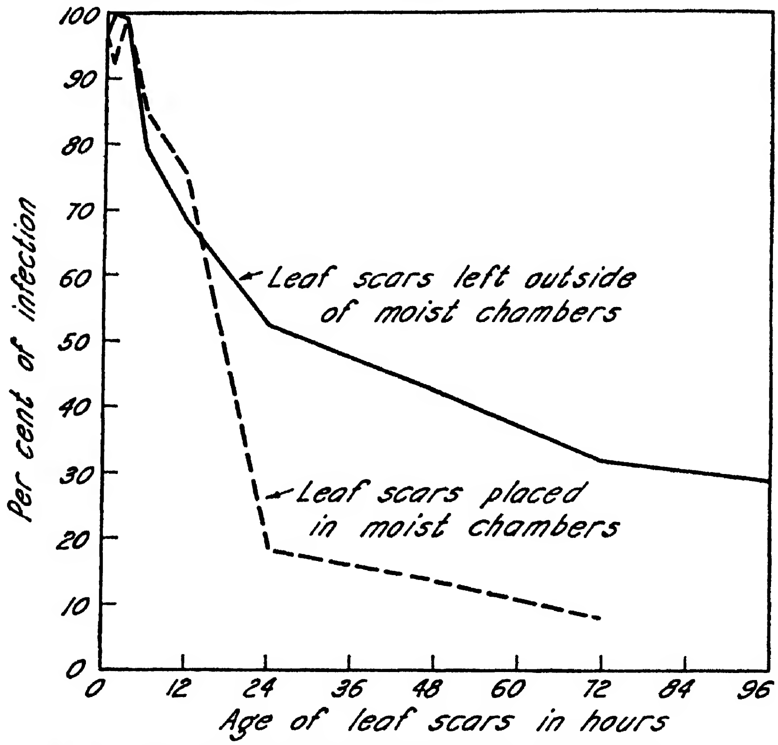

Fig. 5.—Results of the 1937 inoculations of leaf scars at short time intervals after leaf removal, showing the relation between the age of the leaf scars in hours at the time of inoculation and the per cent of scars which developed knots, for both those placed in moist chambers and those left outside.

duration, then, in the early summer the processes that prevent infection take place more rapidly during periods of continuous high humidity than during those of low humidity. Under California conditions, however, the spring rains are usually short, generally lasting only a few hours but seldom continuing more than two days. The leaf scars left outside the moist chambers were subjected, then, to the weather conditions that most frequently occur in the spring. Apparently, therefore, 80 to 95 per cent of the leaf scars are susceptible to infection at the time of leaf fall, the percentage of infectable scars drops to about 40 per cent by the fourth day, and most scars are immune to infection by the end of the ninth day after leaf fall.

MICROCHEMICAL STUDIES OF THE HEALING PROCESSES IN LEAF SCARS

Microchemical studies of the healing processes of uninoculated leaf scars were made to determine the processes which take place in these tissues and which may explain the results obtained in the inoculation experiments. For this purpose leaf scars were formed by removing yellow leaves, but the scars were not inoculated. One group was placed in moist chambers; the other left outside of moist chambers. A collection from each group was made when the leaf scars were formed, and other collections of ten scars each were made on each of the ten days following. The scars were preserved in the alcohol-formalin-acetic acid fixing solution until they were sectioned. The healing processes of the scars used in these studies should correspond to those of the leaf scars used in the inoculation work, for these collections came at intervals corresponding to the inoculation intervals which were made in experiments to determine how long the scars were susceptible to infection.

Longitudinal sections about 15 microns thick were made with a sliding microtome. Microchemical tests consisted of determining the presence of and changes in wound gum, water-soluble gums, lignin, suberin, oil, starch, and tannins. Unless otherwise stated, the microchemical methods used were those described by Rawlins (*5*).

Wound Gum.—Wound gum is defined as a substance often found in vessels of plants adjacent to wounds and in wood invaded by wood-decay fungi. It is insoluble in water and stains red with phloroglucinol in hydrochloric acid (*5*). Haas and Hill (*1*), in a similar description, mention the following properties of wound gum: It does not swell in water; it is insoluble in sulfuric acid and caustic soda; and on oxidation it yields both mucic and oxalic acids. According to Küster (*3*) wound gum is insoluble in alcohol, ether, carbondisulfide, cold nitric acid, and cold aqua regia but soluble in warm nitric acid and in a combination of hydrochloric acid and chlorate of potash.

Table 2 gives the results of microchemical tests in leaf-scar tissues. The formation of wound gum was one of the first and most conspicuous processes observed in the healing of leaf scars. It occurred in the cell walls, intercellular material, intercellular spaces, and lumina of vessels.

The only test that would distinguish wound gum from lignin was the Maule reaction (*5*), which colors lignified tissues light red but does not give a color reaction with wound gum in the leaf scar. Wound gum, therefore, is regarded in this paper as the water-insoluble material that forms

in the leaf-scar tissues, reacts positively to most lignin tests, but does not color with the Maule reaction.

Longitudinal sections of leaf scars killed and fixed immediately after they were formed exhibited no detectable change in the composition of the tissues except in one scar where a trace of wound gum was found in

TABLE 2

MICROCHEMICAL REACTIONS OF THE WOUND-GUM ZONE IN THE LEAF SCAR

Classifications concluded on Opposite Page

Chemical test	Tissues adjacent to the wound-gum zone				
	Reaction of the cell walls of:				Intercellular material of parenchyma
	Cortex parenchyma	Cortex fiber cells	Xylem parenchyma	Vessels	
Iodine and potassium iodide	—*	Yellow	—	Yellow	—
I-KI followed by 72 per cent H_2SO_4	Blue	Yellow, later brown	Blue	Yellow, later brown	—
Zinc chloriodide	Blue	Light clear yellow	Blue	Light clear yellow	—
Ferric chloride and potassium ferricyanide	Green	Light blue	Green	Light blue	Dark green
Dinitro-phenylhydrazine	—	Yellow	—	Yellow	—
Maule reaction	—	Light red	—	Light red	—
Phloroglucinol in HCl	—	Red	—	Red	—
Orcinol followed by HCl	—	Light blue	—	Light blue	—
Ruthenium red‡	—	—	—	—	—
Phloroglucinol in HCl‡	—	—	—	—	—
Zinc chloriodide‡	Blue	Blue	Blue	Blue	—
Ruthenium red¶	Light red	Red	Red	Light red	Red
Phloroglucinol in HCl¶	—	—	—	—	—
Zinc chloriodide¶	Blue	Blue	Blue	Blue	—
Polarized light	Anisotropic	Anisotropic	Anisotropic	Anisotropic	Isotropic

* The dashes indicate negative results.
† The substances were removed by chlorination.
‡ After chlorination and sodium-sulfite treatment.
¶ After chlorination and treatment with 10 per cent ammonium hydroxide.

cortical cells. The first change regularly noted after the scars were formed was in the protoplasm of the cells that were to make up the wound-gum zone. Such protoplasm contained a yellow amorphous material (table 4) which stained a dark brown when treated with phloroglucinol in hydrochloric acid.

Traces of wound gum were found at the end of the first day in the tissues of scars left outside moist chambers (table 3 and plate 1, *A*), but not until the second day in the tissues of the scars placed in moist chambers (table 5 and plate 3, *A*). In the former the wound-gum zone developed in 3 to 6 layers of cells, which were usually from 4 to 11 tiers of cells from the surface of the scar. Photographs of stages in the formation of wound gum under these conditions are shown in plates 1, 2, and 3.

Wound-gum zone of the leaf scar

Reaction of the cell walls of:				Reaction of the material plugging the:		Inter-cellular material
Cortex parenchyma	Cortex fiber cells	Xylem parenchyma	Vessels	Lumina of vessels	Intercellular spaces	
Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Yellow, later brown	Yellow, later brown	Yellow, later brown	Yellow, later brown	Yellow, later brown	Yellow, later brown	Yellow, later brown
Deep yellow	Deep yellow	Deep yellow	Deep yellow	Deep yellow	Deep yellow	Deep yellow
Blue	Blue	Blue	Blue	Blue	Dark blue	Dark blue
Deep yellow	Deep yellow	Deep yellow	Deep yellow	Deep yellow	Deep yellow	Deep yellow
——	Very light red	——	Light red	——	——	——
Red	Red	Red	Red	Red	Red	Red
Blue	Blue	Blue	Blue	Blue	Blue	Blue
——	——	——	——	Removed†	Removed	Removed
——	——	——	——	Removed	Removed	Removed
Blue	Blue	Blue	Blue	Removed	Removed	Removed
Light red	Light red	Red	Light red	Removed	Removed	Red
——	——	——	——	Removed	Removed	——
Blue	Blue	Blue	Blue	Removed	Removed	——
Anisotropic	Anisotropic	Anisotropic	Anisotropic	Isotropic	Isotropic	Isotropic

In those scars outside moist chambers wound gum usually began to stain first in the walls of the cortex cells, though in many sections the material was noted in the walls of living cells of the xylem in the region of the wound-gum zone (plate 1, *A*). With an increase in the age of the scars came a corresponding development of the wound-gum zone (tables 3 and 4). The intercellular spaces generally filled about the second day. The plugging of the vessels, which usually began about the third day,

TABLE 3

RESULTS OF STUDIES OF THE HEALING OF LEAF SCARS* THAT WERE FORMED IN MAY AND KEPT OUTSIDE OF MOIST CHAMBERS

Age of leaf scars (days)	Presence of wound-gum layer	Depth of wound-gum layer (number of cells from scar surface)	Plugging of vessels by wound-gum		Amount of starch†			Number of scars developing periderm	Number of cell divisions in periderm	Suberinlike material in cells of the wound-gum zone	Scars having oil in cells of wound-gum zone	Tannins in leaf-scar tissues
			Vessels counted	Vessels plugged	Exterior to wound-gum zone	In wound-gum zone	Interior to wound-gum zone					
0	–‡	–	–	–	+++	+++	+++	–	–	–	–	–
1	T	5 to 11	–	–	+++	+++	+++	–	–	–	–	–
2	+	4 to 12	–	–	+++	++	+++	–	–	–	–	–
3	+–	4 to 12	243	24	+++	+–	+++	–	–	T	–	–
4	++	2 to 12	254	70	+++	+	++	–	–	+	1	–
5	++–	4 to 12	264	204	+++	T	++	–	–	+	–	–
6	+++	4 to 12	–	–	+++	–	+	2	1 to 2	+–	3	–
7	+++	4 to 12	270	269	+++	–	+	16	1 to 2	++	2	–
8	+++	4 to 13		all	+++	–	T	20	1 to 4	++	2	–
9	+++	4 to 10		all	+++	–	T	10	2 to 4	+++	–	–

* Twenty scars were studied at each day group except the ninth day in which 10 scars were used.

† The amount of starch is on a comparative basis

‡ Legend: – = negative; + = positive, +– ++, and +++ = increasing amounts in their respective order; T = trace

was well advanced by the fifth, as shown in table 3 and plate 2, *A*. By the sixth to seventh days the wound gum layer appeared completed in a number of scars (plate 1, *C*, 2, *C* and *D*), and the formation of a periderm had started in a few scars (plate 1, *D* and table 3).

TABLE 4

RESULTS OF STUDIES OF THE HEALING OF LEAF SCARS THAT WERE FORMED IN SEPTEMBER

Leaf-scar number	Age of leaf scar (days)	Wound gum by phloroglucinol in HCl	Orcinol test for wound gum	Depth of wound-gum layer (number of cells from scar surface)	Presence of yellow amorphous material	Copper acetate and ferric chloride test for tannins
Scars left outside moist chambers						
1	0	–*	–*	–*	–*	–*
2	0	–	–	–	–	–
3	0	–	–	–	–	–
4	0	–	–	–	–	–
5	0	–	–	–	–	–
6	0	–	–	–	–	–
7	0	–	–	–	–	–
8	0	–	–	–	–	–
9	0	–	–	–	–	–
1	1	T	T	5 to 8	+	–
2	1	T	T	7 to 10	+	–
3	1	T	T	8 to 11	+	–
4	1	–	–	–	+	–
5	1	T	T	7 to 9	+	–
6	1	T	T	5 to 8	+	–
7	1	T	T	6 to 10	+	–
8	1	T	T	4 to 8	+	–
9	1	T	T	5 to 10	+	
10	1	T	T	6 to 11	+	–
11	1	T	T	6 to 9	+	–
12	1	–	–	–	+	–
13	1	T	T	5 to 8	+	–
14	1	T	T	6 to 11	+	–
15	1	T	T	8 to 12	+	–
16	1	T	T	4 to 9	+	–
17	1	–	–	–	+	–
18	1	–	–	–	+	–
19	1	T	T	7 to 9	+	–
20	1	T	T	9 to 11	+	–
1	2	+	+	4 to 9	+	–
2	2	+	+	5 to 7	+	
3	2	+	+	6 to 9	–	–
4	2	+	+	7 to 10	–	–
5	2	+	+	4 to 8	–	–
6	2	+	+	6 to 9	+	–
7	2	+	+	6 to 10	+	–
8	2	+	+	7 to 9	+	–
9	2	+	+	6 to 8	+	–
10	2	+	+	4 to 9	+	–

* Legend. – = negative; + = positive; +–, ++, +++ = increasing amounts in their respective order; T = trace.

TABLE 4—(*Concluded*)

Leaf-scar number	Age of leaf scar (days)	Wound gum by phloro-glucinol in HCl	Orcinol test for wound gum	Depth of wound-gum layer (number of cells from scar surface)	Presence of yellow amorphous material	Copper acetate and ferric chloride test for tannins
			Scars kept in moist chambers			
1	0	–*	–*	–*	+*	–*
2	0	–	–	–	–	–
3	0	–	–	–	+	–
4	0	–	–	–	–	–
5	0	–	–	–	–	–
6	0	–	–	–	–	–
7	0	–	–	–	–	–
8	0	–	–	–	–	–
9	0	–	–	–	–	–
10	0	–	–	–	–	–
1	1	–	–	–	+	–
2	1	T	–	1 to 3	+	–
3	1	–	–	–	+	–
4	1	–	–	–	+	–
5	1	–	–	–	+	–
6	1	–	–		+	–
7	1	–	–	–	+	–
8	1	–	–	–	+	–
9	1	–	–	–	+	–
10	1	–	–	–	+	–
11	1	–	–	–	+	–
12	1	–	–	–	+	–
13	1	–		–	+	–
14	1	–	–	–	+	–
1	2	T	T	1 to 3	+	–
2	2	+	+	1 to 4	+	–
3	2	+	+	1 to 4	+	–
4	2	+	+	1 to 2	+	–
5	2	+	+	1 to 5	+	–
6	2	+	+	1 to 2	+	–
7	2	T	T	1 to 3	+	–
8	2	+	+	2 to 5	+	–
9	2	+	+	3 to 6	+	–
10	2	T	T	2 to 4	+	–

* Legend: – = negative; + = positive; +–, ++, +++ = increasing amounts in their respective order; T = trace.

Although transverse sections through the wound-gum zone were not very satisfactory for these studies, they show how the wound-gum plugs appear in transverse sections of the vessels (plate 3, *F*).

Two layers of wound gum formed in a number of the scars kept in moist chambers. The first layer usually began to develop in scars collected the second day. Wound gum formed in 1 to 6 rows of cells across the surface of the scar (tables 4 and 5 and plate 3, *A* and *B*) and had generally formed completely by the third to fourth day (plate 3, *C*). About

TABLE 5

RESULTS OF STUDIES OF THE HEALING OF LEAF SCARS* THAT WERE FORMED IN MAY AND PLACED IN MOIST CHAMBERS UNTIL COLLECTED

Age of leaf scar (days)	Presence of wound gum	Number of scars developing two layers of wound gum	Depth of wound-gum layer. (Number of cells from scar surface)		Plugging of vessels by wound gum		Amount of starch†			Number of scars developing periderm	Number of cell divisions	Suberinlike material in cells of the wound-gum zone	Scars having oil in cells of wound-gum zone	Tannins in leaf-scar tissues
			Layer 1	Layer 2	Vessels counted	Vessels plugged	Exterior to wound gum zone	In wound-gum zone	Interior to wound gum zone					
0	–‡	–	–	–	–	–	+++	+++	+++	–	–	–	–	–
1	–	–	–	–	–	–	+++	+++	+++	–	–	–	3	–
2	T	–	1 to 6	–	173	3	+++	+++	+++	–	–	–	–	–
3	+	–	1 to 8	–	203	47	+++	++	+++	1	1	–	1	–
4	+–	3	1 to 5	4 to 10	212	163	+++	+	++	3	1	T	3	–
5	+++	2	1 to 4	4 to 10		all	+++	T	+	2	1 to 2	+	1	–
6	+++	13	1 to 4	4 to 8		all	+++	T	+	2	1	+–	–	–
7	+++	16	1 to 4	4 to 8		all	+++	–	T	3	1 to 2	++	–	–
8	+++	10	1 to 3	4 to 9			+++	–	T	1	1	+++	–	–

* Twenty scars were studied at each day group except the eighth day in which 10 scars were used

† The amount of starch is on a comparative basis.

‡ Legend: – = negative; + = positive; +–, ++, +++ = increasing amounts in their respective order; T = trace.

this time a second layer of wound gum began to appear in a layer of cells from 4 to 10 cells from the surface of the scar, below the first layer (plate 3, *D*). Both layers, however, generally converged at the edges of the scar (plate 3, *E*). By the end of the fifth day, wound gum had plugged all the vessels observed in either the first or second zone.

The striking differences in wound-gum formation between the scars left outside moist chambers and those kept in moist chambers are as follows: (1) The scars left outside moist chambers developed only one wound-gum zone, whereas those kept inside developed two layers. (2) The wound gum was usually detectable by the first day in the scars left outside, but not until the second day in those kept in moist chambers. (3) After it once started, however, wound gum formed much more rapidly in the scars kept inside than in those left outside.

Water-Soluble Gum.—The term is used here to designate gumlike materials that are soluble in water as contrasted with wound gum that is insoluble in water. Water-soluble gum, lignin, and wound gum stain blue when treated with orcinol followed by hydrochloric acid. To distinguish, therefore, between wound gum, lignin, and water-soluble gums, a section was first treated with orcinol and HCl. Next, if positive results were obtained, adjacent sections from the same scar were washed in warm water to remove gums, then treated with orcinol. If the material, other than lignified tissues, staining with orcinol was removed by the water, it was considered to be water-soluble gum. Fresh, unfixed leaf scars were used to determine the presence of this material. These studies were made in scars formed September 9. The results for scars kept in moist chambers are recorded under orcinol in table 4, as are also those for scars exposed to outside conditions. Conceivably, water-soluble gums might be deposited in the cell walls before wound gum appeared; but apparently such was not the case, for no positive tests were obtained.

Lignin.—In all cases, phloroglucinol in hydrochloric acid and the Maule reactions were used to distinguish between wound gum and lignin. The former reacts with both substances, whereas the Maule reaction is positive only when the tissues are lignified.

Lee (*4*) described the abscission of leaves and the healing of leaf scars in numerous species of plants. As to the healing processes of *Castanea sativa* Mill., he states: "Directly after leaf fall—the activity of the cells below the surface of separation is at once shown by the change in the chemical composition of the cell wall. Very gradually these become more or less completely lignified." He does not mention wound gum, but the lignification he describes is probably wound gum, which gives the same reactions as lignin with a number of microchemical tests. If he had used

the Maule reaction, possibly he would have failed to get a positive reaction.

According to results of the present studies of olive leaf scars the vessels, fibers, and a few stone cells were the only tissues of the olive leaf scar that were lignified.

Suberin and Oil.—Sudan III was used to determine the presence of suberin and oil.

In describing the healing of leaf scars of *Castanea sativa,* Lee (*4*) states that when the cell walls of the protective layer undergo "lignification" there is deposited on the inside surface of each cell wall of this layer a fine film of suberin. Lee termed the process "lignosuberization." The protective layer he mentions in the leaf-scar tissue of *C. sativa,* is in a position similar to that of the wound-gum zone in the olive leaf scar.

In the cells of the wound-gum zone of the olive leaf scar was found a material deposited as a thin layer or lamella on the inside surface of the cell walls. This material stained red with Sudan III or IV, was insoluble in alcohol, ether, or benzene, but was soluble in 3 per cent KOH and was isotropic when examined with polarized light. As it did not separate from the cell wall with the protoplasm when the cells were plasmolyzed, it is designated as "suberinlike" material because it apparently has the properties of suberin; but its location in the cell differs from that of suberin, which usually impregnates the wall.

The lamella of suberinlike material began to form after some wound gum had been deposited, and showed first in the 3-day-old leaf scars outside moist chambers (table 3) and in the 4-day-old leaf scars kept in moist chambers (table 5). It continued to develop as the age of the scars increased and stopped about the time the periderm began to form.

Oil globules were found, but not consistently, in either those scars kept in moist chambers or those left outside.

Starch.—Iodine in potassium iodide solution was used for starch determination. In all cases the amount of starch in the cells of the wound-gum zone decreased with increase in the quantity of wound gum (tables 3 and 5).

Tannins.—Sections, after treating with a solution of cupric acetate to precipitate tannins, were rinsed in water and treated with ferric chloride. Tannins stain green, blue, or black. By this method, no difference was noted in the quantity of tannin distributed over the leaf scar at any time during healing.

Periderm Formation.—Observations on the formation of a periderm in the leaf-scar tissue were made when the other healing processes were studied. The results appear in table 3 for the scars left outside moist

chambers and in table 5 for those kept in moist chambers. The periderm developed in the tissue just beneath the wound-gum zone. In the scars left outside, the periderm development was regular. The first cell divisions were observed in two 6-day-old scars. The number of scars in which a periderm had begun to develop increased in each day's collection thereafter. By the eighth day all the scars that were sectioned had completed from 1 to 4 cell divisions in the formation of a periderm (table 3).

The formation of a periderm was not regular in the scars kept in moist chambers. One of the 3-day-old scars, and only one, two, or three of the scars collected each day thereafter, through the eighth day, had started to develop a periderm (table 5). This situation differs from the regular increase in the number of scars that formed periderm in those left outside moist chambers.

From the microchemical studies, one cannot easily explain why the number of scars infected dropped so rapidly during the first two days after the leaves were removed (table 1, and figs. 4 and 5). By the end of two days there were apparently no materials deposited within the tissues of the scars that mechanically blocked them to invasion by bacteria. After two days, however, the deposition of wound gum was concurrent with the decrease in the percentage of scars infected. By the time all the vessels of the scars appeared completely plugged with wound gum, the scars had become immune to infection. This held true both for scars kept in moist chambers and for those left outside.

These studies show that during the first two days other factors may be involved in the resistance to infection.

COURSE OF BACTERIA THROUGH LEAF-SCAR TISSUE TO ESTABLISH INFECTION

The processes of healing in the leaf scars previously described do not explain why the susceptibility of scars to infection decreased rapidly during the first two days. They also do not explain why this drop was much more pronounced in the scars kept in moist chambers than in those left outside. A study was made, therefore, of the channels through which the bacteria could enter the tissues to cause infection.

For these studies, a mixture of 1 part of Higgins' American India ink and 5 parts of water proved most satisfactory because it did not diffuse into the living cells and was insoluble in water after it had dried in the scars. This procedure facilitated handling of the scars in water during and after sectioning.

In the experiment several scars were formed as previously described

under methods. One group was placed under moist chambers; another was left outside. When the leaves were being removed and at intervals thereafter, a few scars in each group were covered with the ink mixture, applied with a small brush. The ink mixture usually remained on the surface of the scar about 10 minutes before drying. After the ink dried, the scars were removed and brought into the laboratory for sectioning. The sections were mounted in water, and the depth of ink penetration was measured with a filar micrometer. The greatest depth of penetration

TABLE 6

THE PENETRATION OF INDIA INK INTO THE VESSELS OF LEAF SCARS

Age of leaf scar at time of treatment (hours)	Scars outside moist chambers			Scars inside moist chambers		
	Number of scars treated	Scars in which ink penetrated 5 cells or more (per cent)	Average depth of ink penetration (microns)	Number of scars treated	Scars in which ink penetrated 5 cells or more (per cent)	Average depth of ink penetration (microns)
0	34	86.3	493.3	34	86.3	493.3
½	30	74.2	154.2	20	70.0	139.4
3	25	70.0	65.3	22	63.5	69.5
12	29	48.3	23.9	25	32.0	20.3
24	30	48.2	10.7	22	27.6	8.2
48	25	27.9	8.0	20	15.0	7.3

in each scar was recorded, and the average for each collection was computed from these measurements (table 6, fig. 6). The results show that immediately after the leaves were removed, the ink placed over the scars moves a relatively great distance back into the vessels of the scars. In scars only half an hour old, however, the ink penetrated only a relatively short distance; and this distance decreased thereafter with increasing age of the scar (table 6, fig. 6, *A*). A comparison of these results with those of infection data (fig. 5) suggests that the reason a high percentage of the scars developed knots when inoculated immediately after removal of the leaves was that the inoculum penetrated deeply into the vessels at this time.

In these observations the ink penetrated only through the vessels; in no case was it found to have entered the intercellular spaces or sieve tubes.

If the formation of wound gum in the leaf-scar tissues prevents the entrance of bacteria, the latter, in order to infect the host, must pass beyond the wound-gum zone before it plugs the channels of entrance. The wound gum developed in 4 to 12 cell layers from the surface of the scar. An average thickness of this zone was around 2 to 5 layers of cells.

Apparently, then, if the initial penetration of the bacteria into the scar tissues was 5 cell-layers or more, they were deep enough in the scar tissues to establish infection.

In these studies of ink penetration, therefore, if the India ink placed over the surface of the scar penetrated the tissues 5 cells or more, it was considered deep enough for bacteria to have caused infection. If this

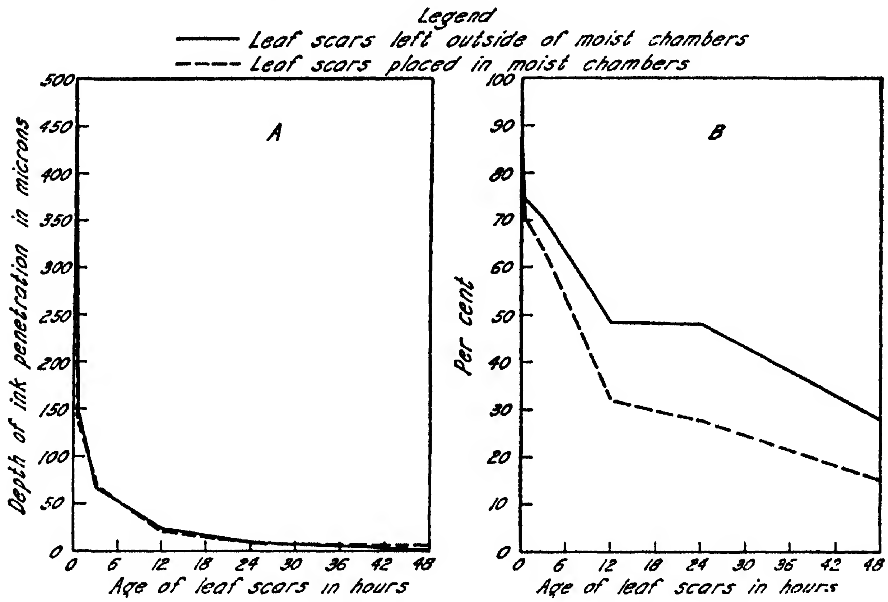

Fig. 6.—The results of studies of India-ink penetration into leaf-scar tissues for scars kept in moist chambers and those left outside. *A*, The relation between the age of the leaf scar in hours when treated with ink and the depth of ink penetration. *B*, The relation between the age of the leaf scars in hours when treated with ink and the per cent of scars in which the ink penetrated to a depth considered sufficient to cause infection if bacteria had been used.

assumption is correct, the percentage of scars in which the ink penetrated 5 cells or more should correspond to the results of inoculation experiments under similar conditions.

The curve (fig. 6, *B*) representing the percentage of scars in which ink penetrated to a depth of 5 cells, or more, compares very closely for the period of the first two days with the results of interval inoculations in the curves of figure 5. For the scars left outside moist chambers the ink penetrated to a depth of 5 cells, or more, in 48 per cent of the scars one day old, and in 28 per cent of those two days old, whereas in the inoculation experiments about 58 per cent of the one-day-old scars developed knots and about 45 per cent of the two-day-old scars developed knots.

For the scars kept in moist chambers the ink penetrated 5 cells or more in about 27 per cent of the one-day-old scars and 15 per cent of the two-

day-old scars. In the inoculation experiments about 23 per cent of the one-day-old scars developed knots, and about 15 per cent of those two days old.

Judging from the ink-penetration studies under the conditions of these experiments, the infection of leaf scars may be influenced by the depth of penetration of the inoculum into the vessels.

THE DEVELOPMENT OF BACTERIA IN THE SCAR TISSUES

The course the bacteria take in establishing infection was studied in artificially inoculated leaf scars. A large number of scars were formed, marked, and inoculated as previously described. Groups of 10 scars each were collected 2, 4, 6, 8, 10, 13, 15, 19, and 26 days after the scars were inoculated. The scar tissues were imbedded in paraffin, as described earlier, and were sectioned with a rotary microtome. The sections were stained with Stoughton's stain for bacteria in tissues (5).

Theoretically the bacteria might infect a leaf scar in several ways: (1) by growth on the surface of the scar, (2) by entering the scar by breaking down the tissue, (3) by penetrating the scar tissue through the intercellular spaces, (4) by entering through fissures in the scar, (5) by entering the scar through the sieve tubes, and (6) by entering the scar tissues through vessels. In the scars examined, the only way, with one exception, in which infection had been established was through the vessels. In one case, however, the bacteria had penetrated deeper than the wound-gum zone through a fissure between cortex parenchyma cells. A photograph of this section is shown in plate 4,*A*. In most cases, according to observations, the bacteria established on the surface, in fissures, and in the intercellular spaces of the leaf-scar tissue are later barred from entering more deeply into the tissue by the formation of wound gum and finally of a periderm.

Bacteria pass deeply into the vessels when the leaf scars are inoculated immediately after the leaves were removed. This point is supported by the experiment in which India ink was placed on the surface of scars and passed freely through the vessels of the leaf and, in a few cases, as deep as the vascular system of the stem. Small groups of bacteria were, furthermore, found scattered along in the vessels (plate 4, *B*) and lodged on the rims at the ends of the vessel cells in sections of scars collected 4 days after inoculation. Bacteria were observed between the secondary thickenings, where they may have lodged as the inoculum was drawn back through the vessels. The groups of bacteria shown in these photomicrographs (plate 4, *B*) are stained very heavily in order that the vessel walls

may be shown. They appear, therefore, only as dark masses lodged between the thickening on the sides of the vessels. These small groups of bacteria enlarge rapidly, producing colonies that finally merge and completely fill the vessels (plate 4, *C*).

The bacteria are freed from the vessels into the other tissues of the leaf scar when the forming periderm pulls the vessels apart (plate 4, *D* and *E*). The periderm forms just beneath the wound-gum zone, and the increase in number and size of the cells pulls the vessels apart at this point. This generally occurs from 8 to 10 days after the scars are formed. The bacteria are then released into the newly forming periderm, a region of actively dividing cells (plate 4, *D* and *E*). The bacteria confined in the vessels deeper in the leaf traces remained there and did not break out into the surrounding tissue by the end of the 19 days after inoculation (plate 4, *E*).

The presence of bacteria or their products among the meristematic cells of the periderm apparently stimulates these cells to active division, and it appears that the successive rows of parenchyma cells were derived from the phellogen (plate 5, *A*). The bacteria increase in numbers at the end of the broken vessels and form small pockets, which increase in size and grow with the surrounding tissue. After 19 days the pockets are fairly large (plate 5, *A*). Some of the cells adjacent to them break down, collapse, and remain around the outer portions of the pockets (plate 5, *B*). As the plate shows, more cell divisions occur in the region around the bacterial pockets.

SUMMARY

A study has been made of certain factors involved in the infection of olive leaf scars by *Bacterium savastanoi* E.F.S. from the stage just before leaf fall until a periderm develops in the scar.

It was found that natural infection in the region of the abscission zone rarely occurs before leaf fall.

Evidence obtained confirms the conclusions of previous workers that most of the new knots forming each year develop at leaf scars.

Most leaf scars were susceptible to infection immediately after leaf fall; the susceptibility dropped rapidly during the first day; and the scars became immune by the end of the ninth. The drop in susceptibility was much more rapid for scars kept in moist chambers than for scars left outside.

Microchemical studies of the abscission processes of leaves and the healing of leaf scars showed that (a) no protective layers are formed in the tissues before leaf fall; (b) separation takes place through the inter-

cellular material between two rows of cells in the base of the abscission zone; (c) the sieve tubes, vessels, cuticle, fibers, and apparently the epidermal cells are mechanically broken at leaf fall; (d) during the healing processes of the olive-leaf scar a wound-gum layer is first formed and is followed by the development of a periderm; (e) water-soluble gums, lignin, oil, suberin, starch, and tannins apparently have no influence upon infection.

India ink was used to trace the course of the inoculum from the surface of the leaf scar into the scar tissues. Judging from these studies, infection may depend upon the depth of penetration of the inoculum.

Most infections in the leaf scars were caused by bacteria that entered the tissues through vessels. Bacteria entering the leaf-scar tissue through intercellular spaces progressed slowly and were stopped by wound gum that plugged these spaces. Those entering the vessels were freed into the periderm cells when the vessels were slowly pulled apart by growth of the periderm. Pockets of bacteria were formed in the tissues derived from the phellogen, and the greatest amount of cell proliferation occurred around the pockets of bacteria.

ACKNOWLEDGMENTS

The author is grateful to Dr. T. E. Rawlins, Dr. E. E. Wilson, and Dr. A. S. Foster for their guidance during the research work; to Professor Max W. Gardner for helpful suggestions and criticism and to the owners of the Cross Ranch in Sacramento County for the use of their orchard.

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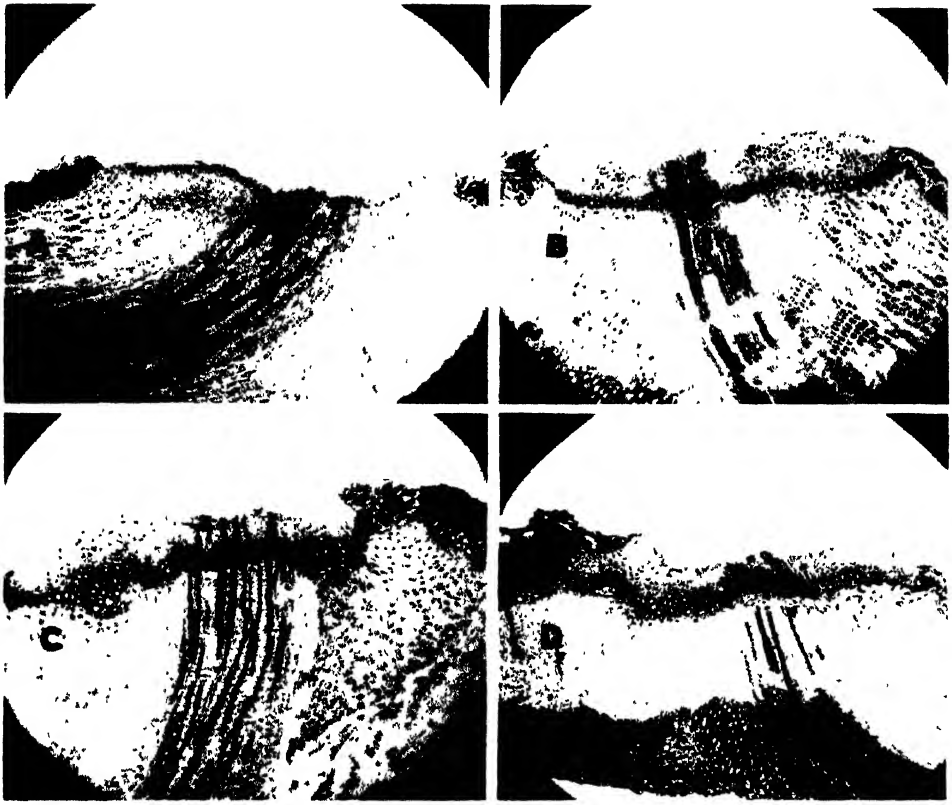

Plate 1.—Sections of leaf scars approximately 15 microns thick. The sections were treated with phloroglucinol in hydrochloric acid and mounted in the same solution. The lignified walls, a portion of the cell contents, and the wound gum were the only parts of the sections that stained with the phloroglucinol in hydrochloric acid; they appear particularly dark in the photographs. Stages in the development of wound gum in leaf scars left exposed to outside atmospheric conditions. *A*, Longitudinal section of leaf scar 1 day old. The wound-gum layer has begun to form at a distance of 5 to 8 cells from the edge of the leaf scar, as indicated by darker staining in the cortex. *B*, Longitudinal section of a leaf scar 5 days old. The section shows the wound-gum layer being formed and wound-gum plugging some of the vessels. Photographs at higher magnifications showing the plugging of the vessels are in plate 2, *A* and *B*. *C*, Longitudinal section of leaf scar 7 days old. All the vessels of the wound-gum area are completely plugged with gum. Photographs of higher magnifications of the wound-gum zone are shown in plate 2, *C* and *D*. *D*, Longitudinal sections of leaf scars 9 days old. From 1 to 3 cell divisions have occurred beneath the wound-gum zone in an early stage in periderm formation. Also note the vessels that have been pulled apart. (All × 36.)

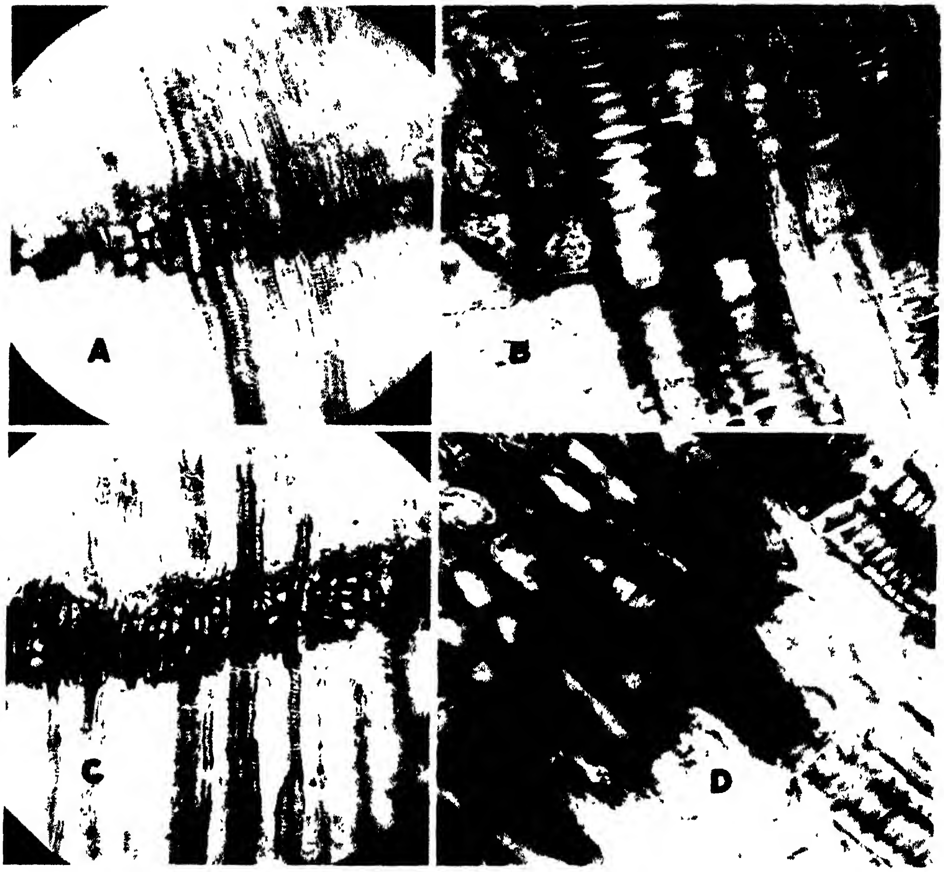

Plate 2.—Vessels plugged by wound gum, and wound gum in the walls of the parenchyma cells. *A*, From the same longitudinal section of a leaf scar as photograph *B* in plate 1. *B*, A portion of the same section as *A*. This shows more clearly the wound gum in the vessels and walls of parenchyma cells. *C*, From the same longitudinal section of leaf scar as photograph *C* in plate 1. *D*, A portion of the same section as *C*. (*A* and *C*, × 192; *B* and *D*, × 694.)

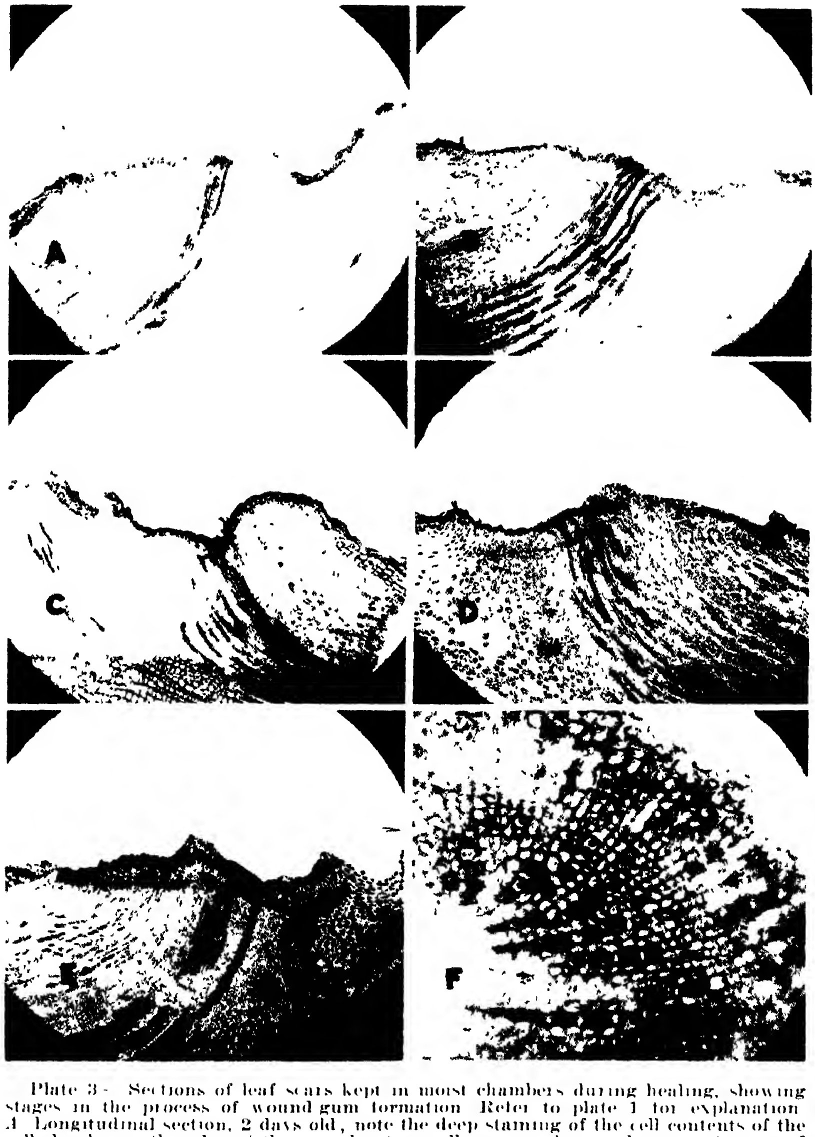

Plate 3.—Sections of leaf scars kept in moist chambers during healing, showing stages in the process of wound-gum formation. Refer to plate 1 for explanation. *A*, Longitudinal section, 2 days old; note the deep staining of the cell contents of the cells bordering the edge of the scar due to a yellow amorphous substance. A trace of wound gum has developed in the upper right edge of the scar. *B*, Longitudinal section, 2 days old, showing a trace of wound gum along the left side in the cells bordering the edge of the scar. *C*, Longitudinal section, 4 days old. The wound-gum layer borders the entire edge of the leaf scar and has completely plugged all vessels and intercellular spaces. *D*, Longitudinal section, 7 days old. The second layer of wound gum has begun to form 3–5 cells interior to the first wound-gum layer. *E*, Longitudinal section, 8 days old. The second layer of wound gum is formed completely. *F*, A portion of a transverse section through the wound-gum zone of a leaf scar 7 days old, showing wound gum plugging some of the vessels. (All × 36.)

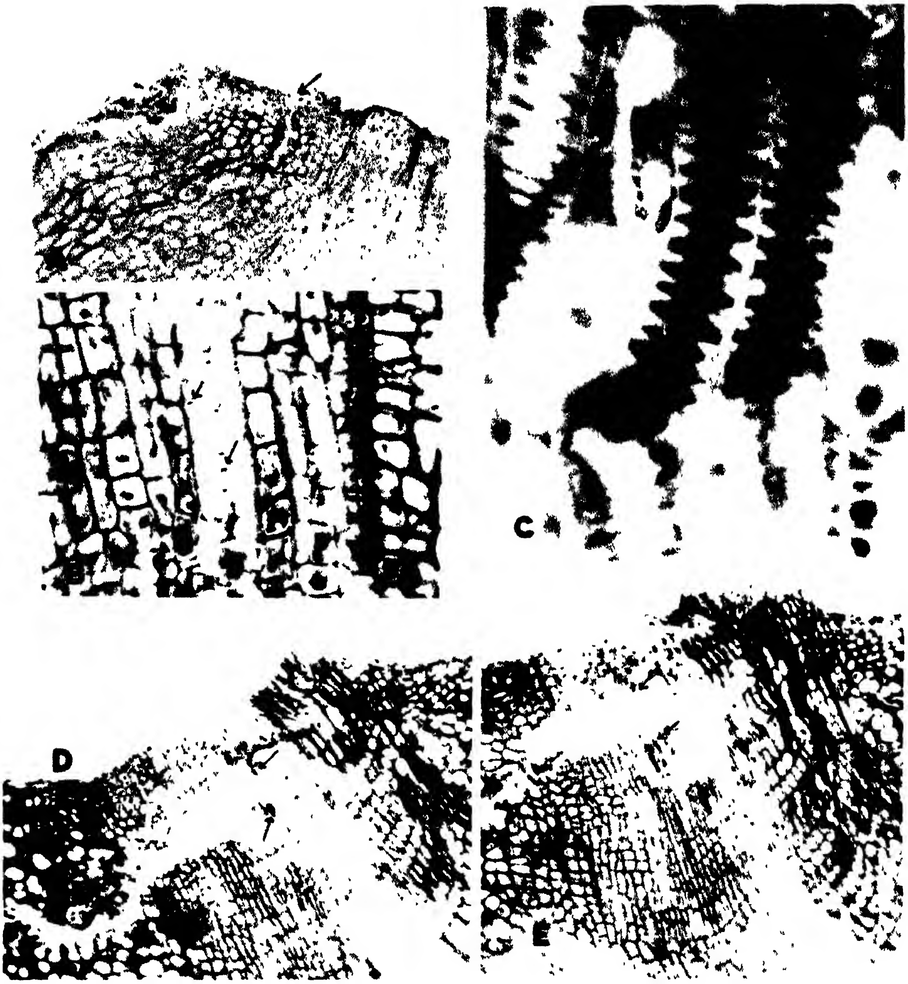

Plate 4.—A, Longitudinal section of a leaf scar 12 days old. The leaf scar was inoculated immediately after the leaf was removed. The upper arrow indicates a fissure between the cortex cells through which infection took place, and the lower arrow points to a colony of bacteria. B, Vessels from a longitudinal section of leaf scar 4 days after inoculation. The vessels contain colonies of bacteria between the secondary thickenings of the wall, as indicated by arrows. C, Vessels from a longitudinal section of a leaf scar collected 7 days after inoculation. They are partly filled with bacterial colonies. The portion of the vessels shown are located just below the wound-gum zone. D, E, Longitudinal sections of leaf scars collected 12 days after inoculation. Note portions of vessels filled with bacteria and pulled apart and separated by the active division of the periderm cells. The walls from the cells derived from the periderm are not clearly stained. (A, × 74; B, × 304; C, × 1004; D and E, × 109.)

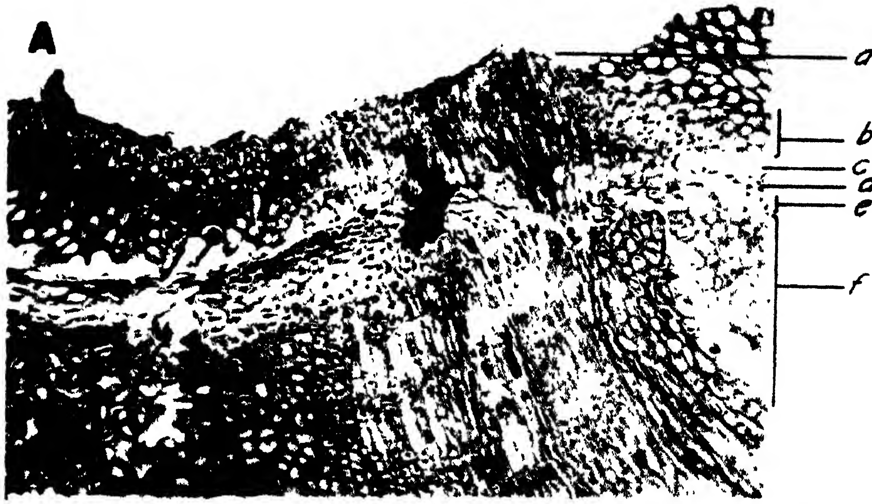

Plate 5. *A*, Longitudinal section of a leaf scar collected 16 days after inoculation. Note the pocket of bacteria formed among the cells of the periderm. The bacteria in the upper portion of the pocket are extended into vessels. *B*, Longitudinal section of a leaf-scar collected 26 days after inoculation, showing large pockets of bacteria which have developed in the tissue apparently derived from the phellogen. Details are: *a*, leaf-scar surface; *b*, region of the wound-gum zone; *c*, phellem; *d*, phellogen; *e*, tissue derived from the phellogen; *f*, leaf petiole tissue. (All × 133.)

CHARCOAL ROT OF SUGAR BEET

C. M. TOMPKINS

CHARCOAL ROT OF SUGAR BEET[1, 2]

C. M. TOMPKINS[3]

INTRODUCTION

Charcoal rot of sugar beet (*Beta vulgaris* L.), caused by *Macrophomina phaseoli* (Maubl.) Ashby, was found in August, 1932, in Sutter County in the Sacramento Valley and subsequently near Davis, Marysville, Walnut Grove, and on Victoria Island in the delta region west of Stockton. The incidence of infection in numerous fields ranged from 8 to 30 per cent (*5*).[4] Field observations indicated that the fungus attacks half-grown and mature sugar beets during the season of prevailing high temperatures and is probably confined to the hot, interior valleys. It is not known to cause damping-off of sugar-beet seedlings. Inspection of sugar-beet plantings in the cool, coastal valleys has shown them to be free from infection.

In studies of a seedling blight of beans (*Phaseolus vulgaris* L.) caused by this fungus, Kendrick (*3*) showed that the disease was favored by high temperatures. Later, under controlled conditions, Tompkins and Gardner (*6*) corroborated Kendrick's results and found that the fungus from charcoal rot of sugar beet grew throughout a temperature range of 12° to 37° C, with an optimum at 31° and was pathogenic to bean seedlings at high temperatures.

A brief discussion on symptoms of the disease, the causal organism, and pathogenicity of the fungus is presented in this paper.

SYMPTOMS OF THE DISEASE

The leaves of diseased plants show pronounced wilting and eventually turn brown and die. Dead leaves remain firmly attached to the crowns. When an infected plant is pulled from the soil, the symptoms of charcoal rot are distinctive enough to readily differentiate it from all other known root rots of sugar beet. Externally, infection is usually confined to the crown region as indicated by brownish-black[5] lesions of irregular size and shape (fig. 1) and with a silvery sheen. On old lesions the periderm is very thin, papery in texture, and loosely attached to the underlying

[1] Received for publication February 11, 1938.

[2] The writer is indebted to Dr. M. W. Gardner for advice and assistance.

[3] Assistant Plant Pathologist in the Experiment Station.

[4] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

[5] In determination of root discoloration, Ridgway's (*4*) system of color nomenclature has been followed.

tissues. Under slight pressure it cracks and becomes detached from the root, exposing dry, black, carbonaceous masses of sclerotia (fig. 1).

When examined in cross section soon after infection, the outer or advancing part of a lesion is mustard yellow but later the inner or older

Fig. 1.—Natural infection of sugar beet by *Macrophomina phaseoli*; an advanced stage of infection, showing black lesions with a silvery sheen, which completely involve all of the crown and most of the taproot tissues. The thin, papery periderm has been partly ruptured on the crowns, exposing masses of black sclerotia directly beneath.

part changes to buffy citrine. These colors merge irregularly into each other, with no sharp line of separation (fig. 2). Occasionally the infected tissues may be a uniform buffy citrine. The advancing margin of a lesion, next to apparently healthy tissues, is undifferentiated in color from the tissues invaded earlier and has no distinctive dark band such as characterizes the root rot of sugar beet caused by *Phytophthora drechsleri* Tucker (*7*, fig. 2, *C*). After the entire root has become invaded, the tissues

turn in color from buffy citrine to old gold and finally to brownish black. In the late stages of decay, masses of black sclerotia largely displace the periderm and parenchymatous tissues, forming in pockets or cavities of

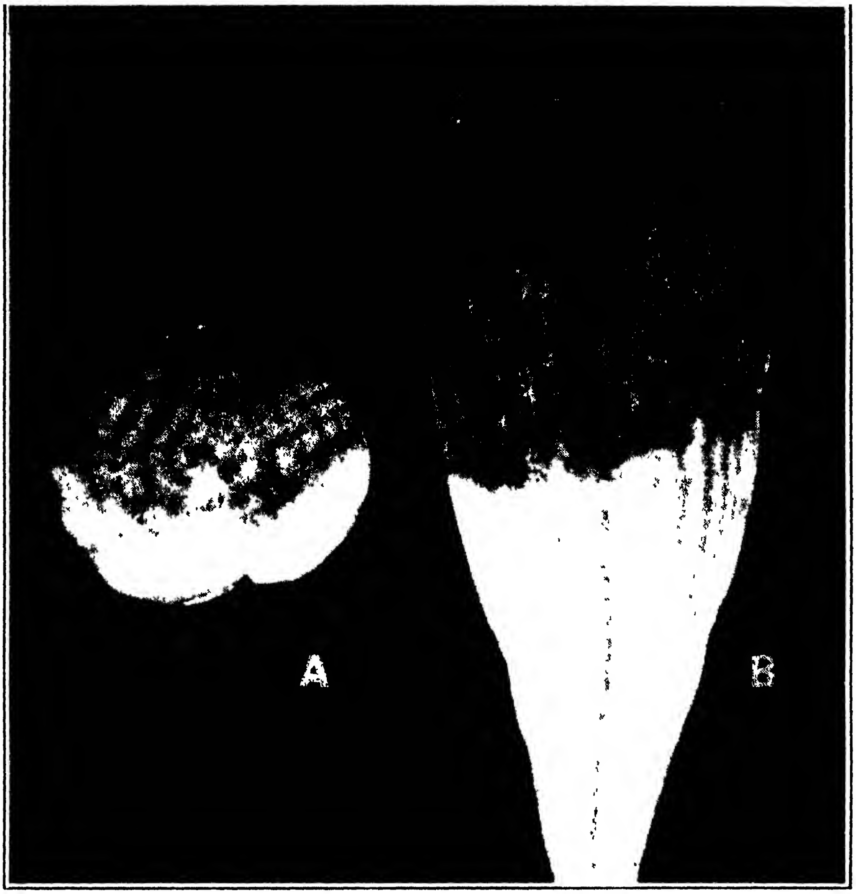

Fig. 2.—Natural infection of sugar beet by *Macrophomina phaseoli: A,* cross section of diseased taproot; *B,* longitudinal section of diseased taproot showing a large mass of black sclerotia at the crown.

irregular size and shape immediately beneath the periderm and extending inward for several centimeters; they may also be found scattered irregularly throughout the mustard-yellow tissues, in marked contrast. Eventually, sclerotial masses occupy the pith and only the vascular elements retain their identity (fig. 3). Completely invaded sugar beets shrink, tend to become mummified, and are of no value for extraction.

Microscopic examination of sections of the infected tissue from inoculated roots stained with magdala red and fast green showed that the mycelium of the fungus was confined to the intercellular spaces.

THE CAUSAL FUNGUS

Tissue fragments from the advancing edge of lesions on approximately 200 sugar beets from various localities were planted on prune agar in petri dishes which were then incubated at room temperature. Colonies containing colorless mycelium usually developed within 24 hours, with but scanty aerial growth. In 36 hours, the medium was darkened by the

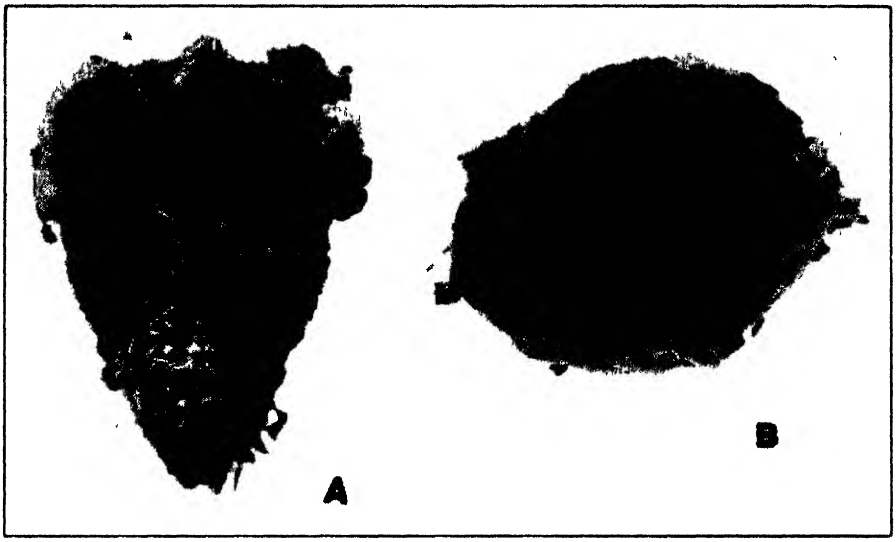

Fig. 3.—Natural infection of sugar beet by *Macrophomina phaseoli: A*, longitudinal view and, *B*, top view of crown, showing symptoms typical of the final stages of decay. The periderm has been completely ruptured and, with the parenchymatous tissues, largely destroyed, exposing vascular elements and masses of black sclerotia. Heavy shrinkage and mummification are not uncommon.

formation of sclerotia which in general were small, spherical, and black and rather evenly distributed. Pure cultures were established on prune agar slopes in test tubes by mass transfers of mycelium and sclerotia.

Six isolates of the fungus from diseased sugar beets collected near Stockton, Sutter Basin, and Walnut Grove were grown on potato dextrose agar, pH 5.6, and incubated at 28° for 14 days. A total of 100 sclerotia from each isolate were measured. The diameters of sclerotia ranged from 46.2 to 146.3 microns, with a mean diameter of 73.8 to 87.2 microns. These isolates, therefore, fall within the limits of Haigh's C group, in which the diameter of the sclerotia is 120 microns or less, and, according to the work of Ashby (*1*) and of Haigh (*2*), should be designated as *Macrophomina phaseoli* (Maubl.) Ashby, although no isolate of the fungus from sugar beets has produced any pycnidia.

PATHOGENICITY OF THE FUNGUS

Healthy sugar-beet roots were washed in tap water, rinsed in three changes of sterile distilled water, and allowed to dry. Two small areas on opposite sides from the root sutures were washed with 95 per cent alcohol, after which small cubes of tissue, averaging $\frac{3}{16}$ inch in size, were removed with a flamed scalpel. Small squares of prune agar containing

Fig. 4.—Artificial infection of sugar beet by *Macrophomina phaseoli;* cross sections of roots almost completely invaded by the fungus. The black areas on opposite sides represent the sites of inoculation wells and are packed with masses of black sclerotia. A small amount of healthy tissue remains in the centers of the cross sections.

new growth of mycelium and sclerotia were then inserted into these wells, after which the openings were covered with adhesive tape to prevent desiccation. Cultures from Walnut Grove, Sutter Basin, and Stockton, respectively, were tested for pathogenicity. Noninoculated controls received the same treatment except that sterile prune agar was substituted for the inoculum.

After inoculation, all sugar beets were placed in large moist chambers consisting of 5-gallon tin cans with pie tins for covers. Each can was provided with a wire-mesh platform to prevent contact of the roots with the water in the bottom of the can. The inoculated sugar beets were incubated at room temperature (20° to 23° C). After 42 days, 32 roots of 37 inoculated with the Walnut Grove culture, 8 roots of 9 inoculated with the Sutter Basin culture, and 3 roots of 4 inoculated with the Stockton cul-

ture, became infected (fig. 4). The noninoculated controls remained healthy. The fungus was reisolated in pure culture from all diseased sugar beets. The reisolated fungus proved pathogenic upon inoculation into healthy sugar beets.

Six sugar beets were inoculated by placing inoculum on the unwounded periderm under aseptic conditions and were held in moist chambers. After 15 days, 5 roots were infected and the fungus was reisolated. This suggests that the fungus may penetrate the unwounded periderm.

Isolates of the fungus from bean (*Phaseolus vulgaris* L.) var. Red Mexican, tuberous begonia (*Begonia tuberhybrida* Voss), cotton (*Gossypium hirsutum* L.), strawberry (*Fragaria* sp.), and sweet potato (*Ipomoea batatas* Poir.) proved highly pathogenic to sugar-beet roots within 15 days after wound inoculations were made. The symptoms produced by these isolates in sugar-beet roots were identical with those resulting from inoculation with the isolates from sugar beet.

The susceptibility of sugar-beet seedlings to infection was tested in paraffined paper cups containing autoclaved sand to which the fungus was added (*6*). After 7 days, it was observed that the fungus attacked cotyledons as well as the roots and stems of seedlings. At room temperature, only 3 of 56 seedlings inoculated were infected; at 25° C, 3 of 53 seedlings; at 28°, 9 of 31 seedlings; at 31°, 21 of 25 seedlings; and at 34°, 8 of 13 seedlings. Controls at each temperature continued healthy. The fungus was readily isolated from diseased seedlings and again proved pathogenic upon inoculation. These results show that higher temperatures are especially favorable to infection.

SUMMARY

A crown rot of sugar beet, caused by *Macrophomina phaseoli* (Maubl.) Ashby, is described.

The disease occurs only in the interior valleys of California and is apparently dependent upon high temperatures.

Infection of sugar-beet roots and seedlings was obtained in the laboratory with different isolates of the sclerotial form of the fungus from sugar beet.

Infection of sugar-beet roots was also obtained in the laboratory with isolates from other hosts.

The optimum temperature for growth of one of the isolates from sugar beet was shown to be approximately 31° C.

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H I L G A R D I A

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THE DEPOSIT OF AQUEOUS SOLUTIONS AND OF OIL SPRAYS[1, 2]

W. M. HOSKINS[3] AND Y. BEN-AMOTZ[4]

INTRODUCTION

THE VALUE OF A SPRAY OIL as an insecticide is largely dependent upon the amount of oil deposited when an emulsion containing the oil is sprayed upon an insect or upon the surface of a plant infested by the insect. Care regarding the quality of the oil, uniformity of the emulsion, and thoroughness of application is obviously of little use if the deposition of oil over the sprayed surface is insufficient, excessive, or uneven.

Previous workers who have studied the behavior of spray-oil emulsions have laid emphasis upon various properties, such as surface tension of the aqueous phase, interfacial tension between the two phases, stability of the emulsion, size of the oil droplets, angle of contact formed when the emulsion or its aqueous phase is placed upon a solid, and other analogous properties. The experiments which have been in progress in this laboratory for several years have led to the opinion that the chief importance of these properties for the deposit of oil lies in their effects upon the relative ease with which the aqueous and the oil phases make and maintain contact with the surface sprayed. During the application of an oil emulsion to a solid, and for some time thereafter, there is competition for room upon the surface of the solid. If the aqueous phase either makes contact everywhere first or is able to displace oil which has reached the surface, the end result will be little or no deposit of the oil. On the other hand, if all the oil makes contact as fast as the emulsion is applied, the deposit will be pro-

[1] Received for publication May 18, 1938.

[2] This article is the fourth of a series having the general title: *Factors Concerned in the Deposit of Sprays.* For earlier numbers see Hensill and Hoskins (1935); Hoskins and Wampler (1936); Ben-Amotz and Hoskins (1937) in "Literature Cited" at the end of this paper.

[3] Associate Professor of Entomology and Associate Entomologist in the Experiment Station.

[4] Graduate student, University of California, August, 1933 to May, 1937.

portional to the duration of spraying; and excessive or deficient amounts will be left on various regions according to how the spray is applied. The desired condition lies between these extremes, and the possibility of obtaining a satisfactory deposit of oil depends upon the correct adjustment of the ability of each phase to make the necessary contact with the solid which is sprayed. The object of the work to be reported here was to acquire information concerning the effects which certain water-soluble substances have upon the behavior of the aqueous and oil phases of spray emulsions as they are applied to the standard surface of beeswax which has been used in previous studies in this laboratory (Hensill and Hoskins, 1935; Ben-Amotz and Hoskins, 1937).[5]

The theory that during the application of an oil emulsion each liquid phase is acted upon by forces which result in displacement of one by the other to varying degrees and that the final amount of oil left as a deposit upon the surface is a resultant of the action of all the components of the emulsion has not been stressed by either physicists or entomologists who have worked with oil emulsions. More particularly, the behavior of the nonoil components of emulsions during the act of spraying has been neglected. Since the final result is necessarily influenced by the behavior of each part of the emulsion, a desirable means of study is the examination of the separate components under conditions which resemble as nearly as possible those which prevail during application of the whole emulsion. Such a plan was followed in the present work.

This paper includes an account of what happens to the water and the emulsifying and wetting agents in oil emulsions as well as to the oil itself during and shortly after spraying. From this information, an attempt is made to interpret oil deposit in terms of certain properties of the components taken both separately and together.

For both theoretical and practical reasons, measurements of the properties of spray liquids under static conditions are of little value as compared with measurements made under the conditions which prevail during application of the sprays, that is, under dynamic conditions. This principle cannot be consistently followed in studying all the properties of sprays: for example, no truly dynamic method for measuring the stability of emulsions is available as yet. As far as possible, the experiments with spray liquids reported in this paper have been conducted under conditions which at least approximate those of actual use. In certain cases, the corresponding behavior under static conditions was studied for comparison.

[5] See "Literature Cited" at the end of this paper for complete data on citations, which are referred to in the text by author and date of publication.

The general scope of the investigation covered the following points: behavior of water and of aqueous solutions of the accessory substances when sprayed upon a surface, the amount of each deposited, the amount of accessory substances deposited, their distribution in the system, the angle of contact during spraying and at rest, the effect of rolling, the behavior of oil emulsions when sprayed, the wetting and spreading of the two phases, the effects of competition for space upon the solid surface, and other factors influencing the amount of oil deposited.

THEORIES OF BEHAVIOR OF LIQUID SPRAYS

The theoretical basis for interpreting the behavior of two liquids sprayed together upon a solid surface lies in the magnitudes of the various interfacial energies and in the mechanical effects resulting from violent impact of the liquids upon the solid. The behavior of a liquid upon a solid horizontal surface has been discussed in detail by so many writers—for example, Rideal (1926) and Adam (1930)—that only certain special features need to be mentioned here. In applying an insecticidal or fungicidal spray, the intent is to reach all parts of the solid surface concerned; as far as this objective is achieved, the surface, at least while the spraying continues, becomes covered with a film of the liquid.

If the liquid is either a pure compound or a solution, its behavior after spraying has ceased depends upon whether the sum of the liquid-surface energy, γ_l, and the solid-liquid interfacial energy, γ_{ls}, is smaller or larger than the solid surface energy, γ_s. If $\gamma_l + \gamma_{ls} < \gamma_s$, the liquid will remain in a film over the surface of the solid; but if $\gamma_l + \gamma_{ls} > \gamma_s$, it will recede from the surface and gather into drops, whose area will be subject to the equilibrium condition that:

$$\gamma_s = \gamma_{ls} + \gamma_l \cos\theta, \qquad 1$$

in which θ is the equilibrium angle enclosed by the liquid at the edge of the drop. In spraying natural surfaces, variations in behavior are often noted between different regions of the same leaf or fruit. These are sometimes due to real differences in the nature of the surface at different points and sometimes to the presence of foreign material, such as dust. If finely divided material which is wet but poorly by the spray is on the surface, real contact may not be made with the surface; and the liquid will retreat very rapidly when spraying has ceased. In the special case when $\gamma_l + \gamma_{ls} = \gamma_s$, the extent to which the liquid film extends will be particularly sensitive to mechanical disturbances. This condition is likely to occur only in certain regions rather than over any entire natural surface.

The introduction of a second liquid phase into the spray leads to greater or less contact of the new liquid with the solid surface and hence to competition for space between the two liquids and ultimately to partial or complete replacement of one liquid by the other. The energies concerned in these processes are those residing in the surfaces of the two

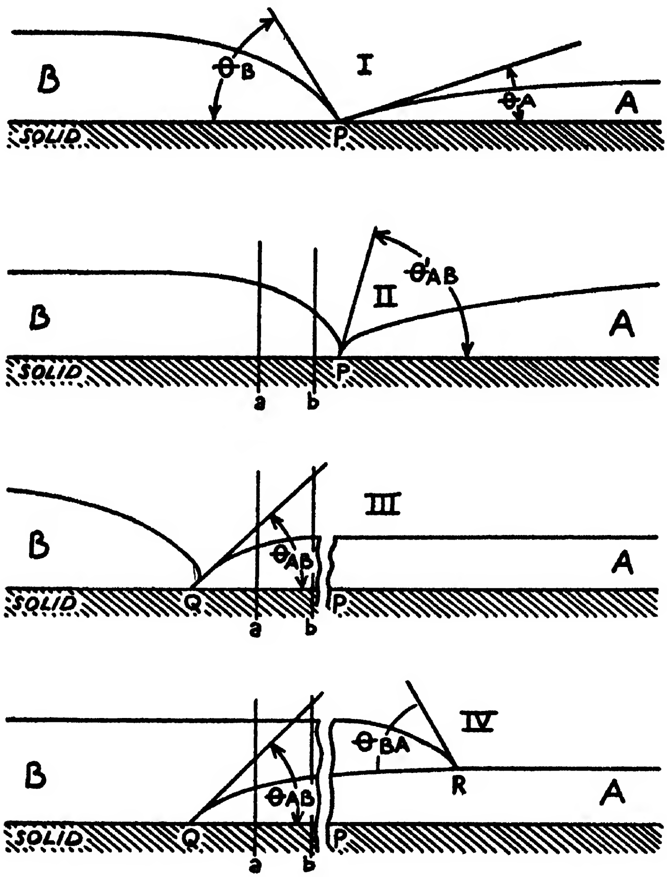

Fig. 1.—Displacement of one liquid from a solid surface by a second liquid.

liquids, in the interface between the liquids, and in the interfaces between each liquid and the solid. In figure 1 are shown diagrammatically certain of the situations which have been observed when limited volumes of oil *A*, and aqueous solution *B*, were applied gently to adjacent areas of a surface. In section I, the advancing front of liquid *A*, which makes a small contact angle, θ_A, upon the solid *S*, meets liquid *B*, which is advancing in the opposite direction and which makes a larger contact angle θ_B. In section II, the depth of both liquids at the line of contact has increased,

and the interface between the two liquids has been established so that it makes the angle θ'_{AB} with the solid surface. This usually is not the equilibrium angle, and the system will change to a condition such as III or IV. Since liquid *B* has been replaced by liquid *A*, the changes in energy per square centimeter of solid surface in III, as in region *a–b*, are $(\gamma_A + \gamma_{SA}) - (\gamma_B + \gamma_{SB})$, which may be called the "replacement coefficient" for any case in which one liquid is pushed back by another and the one which is replaced does not spread over the other. Since limited volumes were assumed for each liquid, obviously the one which spreads will soon be in a very thin layer while the thickness of the other will become greater. At length an equilibrium will be reached whose geometrical characteristics will depend on whether contact is made upon a flat surface, within a trough, within a capillary tube, etc., but which will be characterized by an equilibrium angle θ_{AB} between the solid and the plane of contact of the two liquids where it meets the solid as at point *Q*. When equilibrium has been established, the relations at the line of maximum extension of liquid *A* will be expressed by the equation:

$$\gamma_{SB} = \gamma_{SA} + \gamma_{AB} \cos \theta_{AB}. \qquad 2$$

The situation in which the displaced liquid spreads over the other is represented in section IV of figure 1. The energy change per unit area is $\gamma_B + \gamma_{AB} + \gamma_{SA} - (\gamma_B + \gamma_{SB})$, or $\gamma_{AB} + \gamma_{SA} - \gamma_{SB}$. The equilibrium condition will satisfy equation 2 and θ will have the same value as θ in III unless the layers of liquid are so thick that gravity has an appreciable effect. Liquid *B* may not spread entirely over liquid *A* but instead may assume an equilibrium angle, θ_{BA}, upon it. The equilibrium conditions at *R* may be expressed by the same kind of equation as for a liquid spreading upon a solid, in this case:

$$\gamma_A = \gamma_{AB} + \gamma_B \cdot \cos \theta_{BA}. \qquad 3$$

Only under certain conditions is the above discussion rigidly valid for the behavior of an oil emulsion applied to a solid. The more important of these conditions are: (*a*) the surface is horizontal; (*b*) the effects of gravity are negligible; (*c*) the force with which the liquids strike the surface can be neglected; and (*d*) the effects due to emulsifying and wetting agents present in the emulsion are those for equilibrium conditions. Obviously in many situations none of these limitations hold. The numerous discrepancies between theory and fact in the use of sprays are largely due to the false assumption that an ideal system is being studied. In later sections, the practical effects of certain of the factors listed above will be discussed in detail.

In a system involving water, oil, a solid, and a soluble surface-active substance, the last-named may obviously exert its effect at any or all three of the possible interfaces—(*a*) water-oil, (*b*) water-solid, and (*c*) oil-solid. As a matter of fact, any actual substance of this nature tends to collect to varying extents at all three interfaces, but a distinction may be drawn between *emulsifying agents,* which primarily affect interface *a,* and *wetting* and *spreading agents,* whose chief effect is upon interfaces *b* and *c.*

The difference between wetting and spreading agents has been discussed by numerous workers, but the terms have not always been distinguished clearly. The various points concerned are covered satisfactorily in the following three sets of definitions: (1) A wetting agent is "any substance which causes a spray fluid to wet the sprayed surface so that 'running up' into drops on the surface is avoided;" a spreading agent is "any substance which tends to cause lenses of spray to spread over those portions of the plant surface which have not been hit directly by the spray" (Woodman, 1930). (2) "A wetting agent is any substance which increases the readiness with which a liquid makes real contact with a solid;" "a spreader is a material which increases the area that a given volume of liquid will cover on a solid or another liquid" (Hensill and Hoskins, 1935). (3) "Wetting properties are defined by the ability to form a persistent liquid-solid interface when excess of liquid is drained from the surface;" "spreading properties are defined by the ability of the liquid to form a liquid-solid interface solely by surface activity over the plane surface of the solid" (Evans and Martin, 1935). In order to avoid the use of long and awkward expressions in referring to materials added to the oil-water system for the purpose of altering its properties in one way or another, the term "accessory substance" will be used when no particular function is being emphasized.

The oil-water interface may be affected by a third component which is predominantly soluble either in oil or in water. Hence, both the stability of an emulsion and the relative ease of contact of oil and water with a solid surface may be affected by both water-soluble and oil-soluble accessory substances. The present work has been limited to those which are predominantly water-soluble. Chemically speaking, such substances will have an affinity for water, that is, the phase in which they are more highly soluble, but in order to be able to concentrate in the water-oil interface, they must possess a chemical group or groups which are soluble in the latter also. All emulsifying agents have an affinity for each of the liquid components of the emulsion. Similarly, a substance which aids a liquid to wet a solid must contain groups which have an affinity for

both the liquid and the solid, respectively. Since solids may or may not be similar in constitution to oils, a substance which is a good emulsifying agent for hydrocarbon oils will have different effects in promoting the wetting of various solids by water. A high emulsifying power is not necessarily accompanied by high wetting power and vice versa. Thus, proteins are effective wetting agents for beeswax surfaces, but are relatively poor emulsifiers for hydrocarbon oils, while soaps possess these properties in the reverse relation.

The extent to which a substance may effect the emulsifying, wetting, or spreading properties of a liquid or mixture of mutually insoluble liquids, such as an oil-water emulsion, is dependent upon the time allowed for it to migrate into the interfaces concerned; for the over-all concentration in the bulk of the liquid is usually low, and the marked effects exerted by surface-active substances are due to their ability to become concentrated in regions of transition from one kind of matter to another. The importance of time as a factor in the behavior of oil sprays was emphasized by Ben-Amotz and Hoskins (1937) and will be further illustrated in subsequent sections of this paper.

MATERIALS, APPARATUS, AND METHODS

Three water-soluble surface-active materials, a soap, blood albumin, and hemoglobin, were used as accessory substances. The soap was a so-called "neutral" powder, U.S.P. sodium oleate. It was finely divided, slightly yellow in color, and dissolved completely to give a practically colorless opalescent solution. A stock solution of known strength, made up fresh from time to time, was used in preparing the various solutions and emulsions.

The blood albumin was a commercial preparation of dried blood proteins known as Mapco Blood Albumin. It was a brownish-red powder, containing approximately 3 per cent of insoluble material. A stock solution was used for preparing the various dilutions.

The hemoglobin was prepared in the laboratory from fresh beef blood according to the method of Morse (1927). Great care was taken to avoid conditions likely to cause chemical or physical changes in the hemoglobin, and the various preparations were kept at 0° C in aqueous solution. The concentration of each stock solution was determined by analysis for nitrogen and for iron. In each case the two methods agreed within 1 per cent.

The accessory substances were used at concentrations varying from zero to 0.0225 per cent by weight. For convenience, actual measurements were made in milligrams per gallon of spray and are so given in the

graphs. The relations of milligrams per gallon, per cent by weight, and ounces per 100 gallons are as follows:

Milligrams per gallon	Per cent by weight	Ounces per 100 gallons
71	0.0019	¼
142	0.0037	½
284	0.0075	1
568	0.0150	2
852	0.0225	3

The oil used in all experiments was a "white neutral" tank-mix stock oil of 80 seconds Saybolt viscosity, 92 per cent unsulfonatable residue, and 0.85 grams per cc density. It was secured from the refinery at intervals and kept in closed cans to minimize the changes that take place during storage. This oil was used at 2 per cent by volume in all the emulsions.

The water was ordinary Berkeley tap water whose pH is 8–9 and degree of hardness 45–50 p.p.m. $CaCO_3$. The temperature of the spray liquids when ready for use was 16–20° C.

The surface to which the sprays were applied was a commercial white beeswax, which, for application, was dissolved in carbon tetrachloride at 35° C to a concentration of 4.5 per cent by weight. Oil sample bottles 13.6 cm in height and 3.6 cm in diameter (total lateral area 153 sq. cm) and glass plates 3¼ by 4¼ inches were dipped in the wax solution and then set on a rack to dry for 24 hours. A very thin hard coating was left which appeared to be uniform in properties and whose weight was constant thereafter. The bottles and plates were used as soon thereafter as possible, for after a few days the surface became split by fine cracks and behaved differently when sprayed.

Measurements Made under Static Conditions.—Surface tensions were determined with the du Noüy interfacial tensiometer. All measurements were made by pulling the ring upward. In preparing for a test, care was taken to disturb the surface to a minimum extent in order that the experimental result might be an accurate measure of the tension of a surface of the given age.

Angle of contact was determined by placing a small drop of the liquid upon a horizontal waxed plate and photographing it from the side.

Replacement of one liquid by another was studied by placing drops of each very close together and visually following the changes in position after the drops touched.

Emulsifying power was studied by agitating for 1 minute with a milkshake mixer equal volumes of oil and a solution of the chosen accessory substance. The emulsions were then allowed to stand in tall cylinders

and the time required for 50 per cent of the oil to escape and collect at the top was determined.

Measurements Made under Dynamic Conditions.—The *extent of wetting* of a surface as spray is applied to it was determined by taking photographs with 0.001 second exposure (Craig, 1936). By doing this at intervals, the sequence of events can be followed as a spray is applied.

The *shape assumed by drops* as they roll down a vertical or inclined surface was also studied by the photographic method.

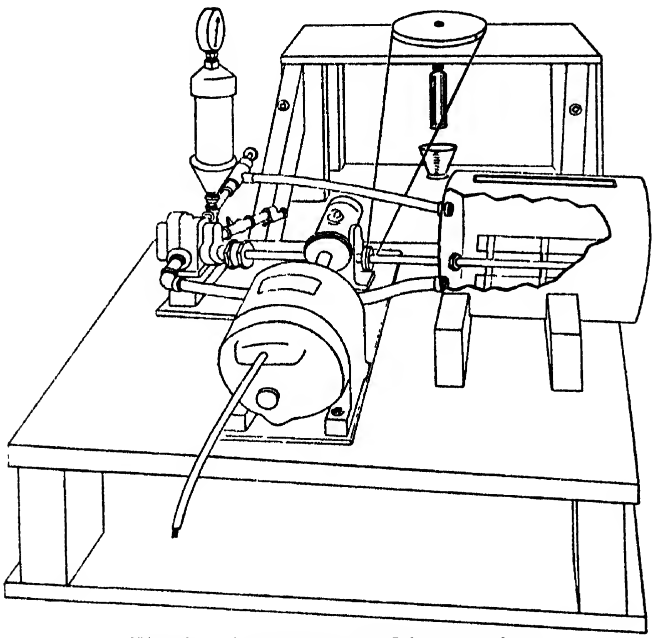

Fig. 2.—Apparatus used in spraying.

The important distinction between the *advancing* and *retreating angles of contact* made by rolling drops is brought out clearly by photographs.

The spraying apparatus consisted of a small gear pump attached to a horizontal 2-gallon tank equipped with an agitator having long flat blades of the ice-cream-freezer type, which revolved 240 times per minute. The nozzle was of the Vermorel design with a disk opening $1/32$ inch in diameter. By properly setting the pin, a solid cone of spray of uniform density can be secured. The objects to be sprayed were placed in a hood 55 cm distant from the nozzle and centered in the spray cone. The bottles were attached by means of a cork to the lower end of a vertical iron rod which rotated at 15 r.p.m. The entire apparatus is shown diagrammatically in figure 2.

The sprays were prepared by putting 1 gallon of water in the tank with the required amount of accessory substance. The agitator was set in motion and oil in the standard amount of 2 per cent by volume was added. After agitation had continued for 5 minutes, the pump was started and the emulsion was recirculated to the tank under a pressure of 80 pounds to the square inch for 1 minute. Sufficient spray was then wasted to flush out the pipes and the waxed bottles were sprayed as rapidly as possible.

The volume of spray applied to each bottle was regulated by catching the runoff in a funnel and graduated tube set closely beneath the bottle. Spraying was continued until the volume of the liquid was 20 cc. At a pressure of 80 pounds per square inch very little liquid is knocked off the bottle, and consequently that which drains from the bottom is a reliable index of the total volume which struck the surface. By means of a shutter set in front of the nozzle, the volume of the spray which reaches the bottle can be controlled to within 1 cc. The corresponding variations in deposit are negligible.

Four bottles were sprayed for each deposit test, and the tests were repeated several times for each condition.

The amount of oil deposited was determined by the difference in weight before and after spraying, 48 hours being allowed for all water to evaporate.

The amount of aqueous solution or of entire emulsion deposited was determined also by the difference between weights before and after spraying, but in these cases the bottles were taken from the spray chamber, placed for 5 minutes in a compartment saturated with water vapor in order that excess liquid might drain off, and then weighed as rapidly as possible.

For studying the amount of accessory substance left upon the surface under various conditions, hemoglobin was used and estimated by analysis for nitrogen by a micro-Kjeldahl method.

RESULTS

Effects of the Accessory Substances upon the Wetting and Depositing Powers of the Aqueous Phase.—The study of the separate components of the emulsions was begun with the aqueous phase since it is the only one in which the surface-active added substances are soluble and which, therefore, may be expected to reveal most simply the effects of their presence.

In figure 3, *A*, the behavior of water and of solutions of blood albumin, hemoglobin, and sodium oleate during application to waxed bottles is shown by photographs taken with 0.001 second exposure according to the method of Craig (1936). When the pictures were taken, spraying had

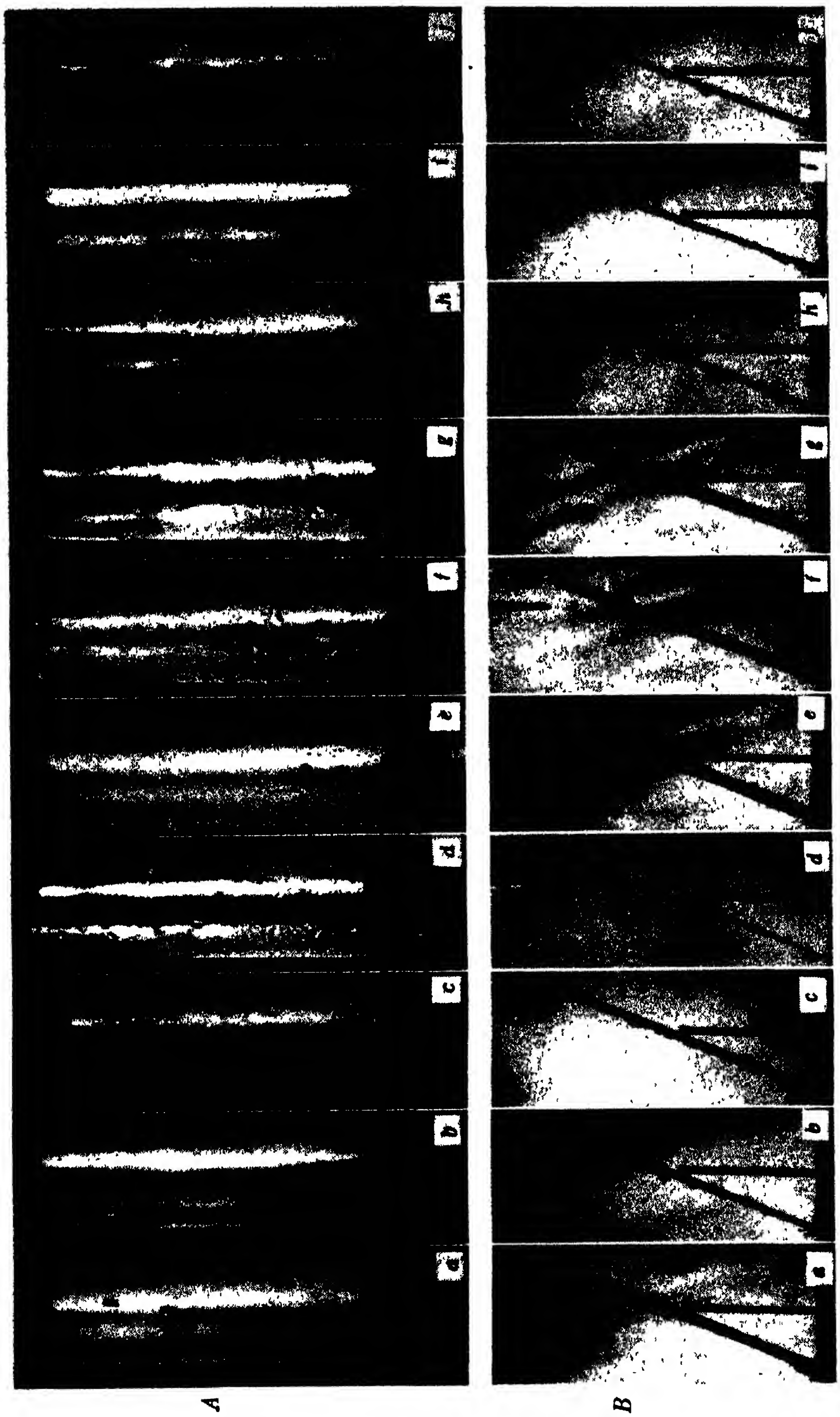

Fig. 3.—*A*, Behavior of aqueous solutions of the accessory substances during spraying; *B*, behavior of drops of aqueous solutions of the accessory substances as they move down an inclined wax surface. Composition of the solutions: *a*, water; *b–d*, blood albumin; *e–g*, hemoglobin; *h–j*, sodium oleate. Each of the accessory substances is in a series of increasing concentrations—35, 142, and 568 mg per gallon.

been in progress for 10 seconds, during which time approximately half the standard runoff of 20 cc had occurred. The appearance at corresponding concentrations of the two protein solutions is somewhat similar and is in sharp contrast to that prevailing with the soap. At equal concentrations, hemoglobin gives somewhat better wetting than the commercial blood albumin. The lowest concentration of each substance causes the solutions to wet but little better than water; but thereafter the proteins cause the formation of broad shallow drops, which coalesce to run down in streams. With the highest concentration illustrated (568 mg per gallon), wetting is fairly well accomplished and broad sheets cover a considerable portion of the area. The soap solution at the highest concentration shown forms only a few narrow streams and no sheets. As a matter of fact, comparable wetting scarcely occurs with solutions of sodium oleate six times as concentrated as those of the proteins.

The contrast between the effects of the two types of accessory substances is illustrated further by photographs taken several minutes after spraying was completed. These conditions are shown in figure 4. The transition from small hemispherical drops to broad shallow drops and eventually to complete coverage of the wax surface by a continuous sheet of solution is shown clearly for the two proteins. On the other hand, even the highest concentration of soap causes only limited formation of streams and no continuous coverage.

The observations and photographs made both during and after spraying leave no doubt that as measured under actual conditions of use, soap solutions are very poor wetting agents for beeswax surfaces, whereas the two protein solutions, particularly the hemoglobin, are excellent for that purpose.

A quantitative study of the amounts of solution left upon the surface was made by allowing each tared bottle to drain for 5 minutes in a chamber practically saturated with water vapor and then weighing it. Very little drainage occurred after this interval.

The results are given in figure 5. They bring out the important fact that whereas the formation of shallow drops tends to increase the amount of liquid remaining upon the surface, as soon as conditions are such that complete wetting occurs, the amount remaining is very greatly reduced. This result is in accord with the finding of Evans and Martin (1935) that the better the wetting the less the maximum amount of spray that could be applied before runoff occurred. Although the conditions of their work and those of the present experiment were very different, in both cases improvement in wetting led to decreased deposit. The soap solution, which does not pass through the state of forming shallow drops but

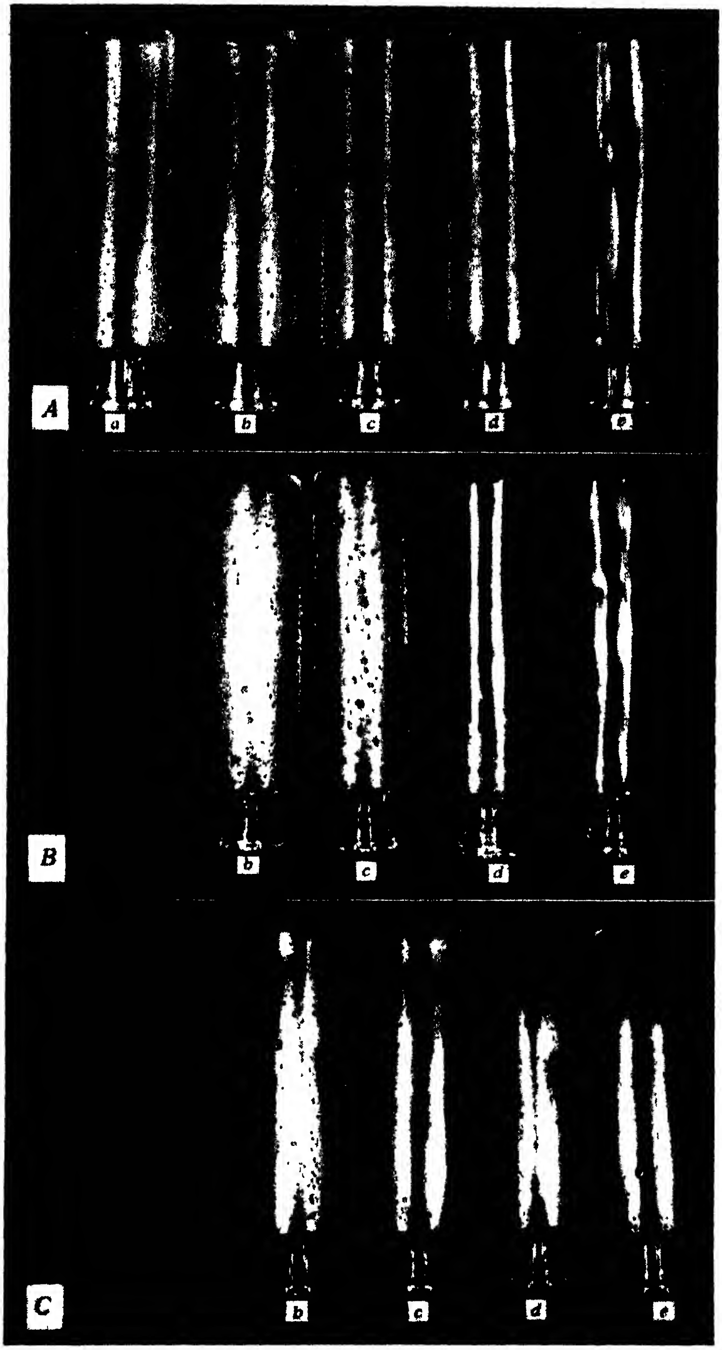

Fig. 4.—Appearance of bottles several minutes after spraying with aqueous solutions of the accessory substances: *A*, *a*, water; *b–e*, blood albumin; *B*, hemoglobin; *C*, sodium oleate. Each of the accessory substances is in a series of increasing concentration—*b*, 71 mg per gallon; *c*, 142 mg per gallon; *d*, 284 mg per gallon; *e*, 568 mg per gallon.

forms very narrow streaks instead, remains upon the surface in continuously decreasing amounts as more solute is used.

It was very easy to notice during the act of spraying that the various solutions made widely differing angles of contact with the solid; to some extent these effects can be seen in the photographs of figure 3, *A*. In order to study this behavior to better advantage, photographs with 0.001-sec-

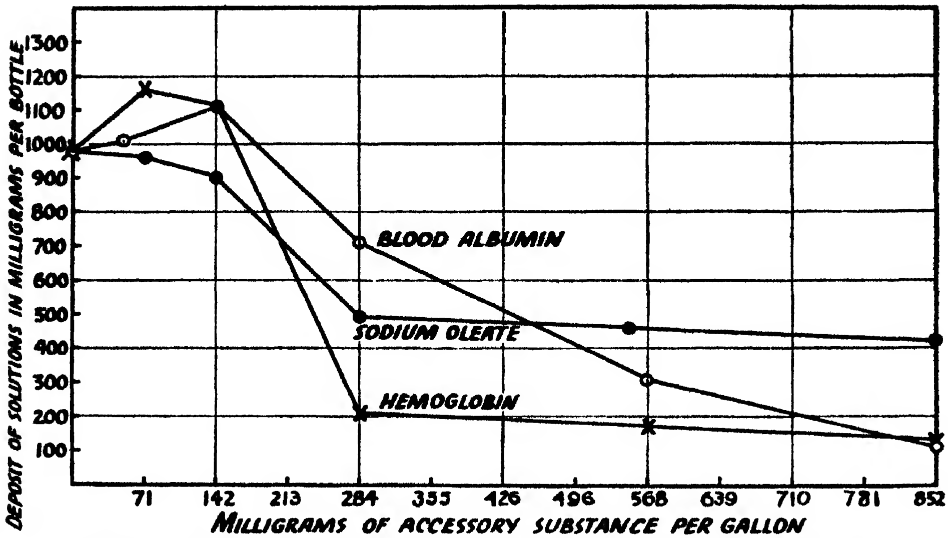

Fig. 5.—Total deposit of solutions of the various accessory substances.

ond exposure were taken of drops of the solutions as they rolled down a waxed slide set at an angle of 24° to the vertical.

The results are shown in figure 3 *B*. Since the drops were made to fall very rapidly from the tip of the burette, they were of nearly uniform size, and several could be photographed at once as they moved down the slide. The following significant differences in behavior of the solutions may be noted: moving drops of the water and of all the soap solutions assume approximately the same shape; but with successively higher concentrations of protein, drops of these solutions flatten, and with the greatest amounts present (fig. 3 *B*, *d* and *g*), a continuous sheet so thin that it cannot be seen from the side is formed by coalescence of the successive drops.

A somewhat similar method of studying spray liquids was suggested by Heranger (1936) who allowed drops to move down the underside of an inclined tube. Under those conditions, the drops are distorted on account of the shape of the solid. For this reason, the present method seems to approximate more closely conditions of normal use of sprays.

The difference between the advancing and retreating angles established during movement of drops down a wax surface is brought out clearly in figure 3 *B*. In the case of water and the soap solutions, the advancing angle is somewhat greater than 90°, whereas the retreating angle is considerably less. However, the important point is not the precise magnitude of these angles but the fact that the latter have a finite magnitude, that is, the drops move as a whole and do not leave a layer of solution behind them. By contrast, the protein solutions, particularly that of hemoglobin, form long tails and, with the higher concentrations, spread from one drop to the next and make a zero retreating angle with the wax surface.

Since this ability of the protein solutions to adhere strongly to the surface is entirely due to the solute, it was of great interest to learn if the proteins become concentrated upon the wax. This was done in the following manner: bottles were sprayed in the usual way, taken from the stand, and shaken several times very vigorously. By this procedure most of the liquid was mechanically removed, and a fairly uniform weight of about 125–150 mg was left as a thin film in all cases. The amounts of protein in these strongly adherent portions of the spray solutions, as calculated from micro-Kjeldahl determinations of nitrogen, are given in columns 2 and 3 of table 1. From the original concentrations in the spray solutions, the amounts of protein in this weight of solution may be calculated approximately, by assuming unit density, to vary from 0.0025 to 0.031 mg. Upon dividing the first and last numbers in columns 2 and 3 of table 1 by these figures, it is found that the blood albumin is from 60 to 25 times as concentrated in the strongly adherent film upon the bottles as in the original solutions and the hemoglobin from 140 to 20 times as concentrated, depending upon whether the weakest or the strongest solutions are compared. As is usual with adsorption processes, the greater relative effect is found with the more dilute solutions.

To gain further information regarding the collection of protein at the wax-liquid interface, analyses were made to determine the amount of blood albumin in all the solutions which remained upon the bottles after spraying, that is, those shown in figure 4. The amounts are given in column 4 of table 1. Comparison of columns 3 and 4 shows that most of the protein remained upon the bottles when they were shaken, though for the lower concentrations at least 80 per cent of the adhering liquid was shaken off. Hence, this experiment also indicates that the protein accumulated at the solid-solution interface.

On account of the emphasis placed by certain writers upon the importance of surface tension as a measure of the wetting or spreading

properties of spray solutions, determinations were made of the surface tensions of the various solutions of blood albumin, hemoglobin, and sodium oleate used in the spraying experiments. All measurements were made upon surfaces 15 seconds old since this is about the least time in which trustworthy tests can be carried out with the du Noüy instrument.

The results are given in table 2. Apparently the differences in behavior of the various solutions during spraying cannot be correlated with the surface tension measured in this way. The tension of older surfaces, such

TABLE 1

WEIGHTS OF PROTEINS IN SPRAY REMAINING UPON WAXED BOTTLES

Concentration of accessory substance in spray liquid	Weight of protein on bottle			
	From solution			From emulsion (not shaken): hemoglobin
	After shaking		Not shaken: blood albumin	
	Hemoglobin	Blood albumin		
1	2	3	4	5
mg per gal	*mg*	*mg*	*mg*	*mg*
71	0.35	0 15	0 25	0 22
142	0 41	0 31	0 41	0.23
284	0 47	0 47	0 66	0 26
568	0 54	0 63	1 14	0.30
852	0 59	0 70	0 70	0 34

as are ordinarily used for such measurement, are even less likely to be related to the wetting power of spray liquids during application.

Effects of the Accessory Substances upon the Behavior of the Oil Phase and of the Complete Emulsion.—The introduction of oil into an aqueous spray liquid leads at once to the situation previously described, that is, competition between the two phases for room upon the surface sprayed. The extent of separation of oil and water during the time an emulsion is passing through the air from nozzle to surface is apparently not known; but since this period is very short with the arrangement used in the present work, most of the surface of the spray drops is probably aqueous. Accordingly, after impact of the spray, there is opportunity for migration of the accessory substance to the solid-aqueous interface, and wetting occurs to an extent dependent upon the concentration of the wetting agent and the nature of the solid.

Photographs of the behavior of various emulsions during spraying are shown in figure 2 of the previous article in this series (Ben-Amotz and Hoskins, 1937). Comparison of these pictures with those for the solutions

containing no oil (fig. 3, *A*, of this paper) reveals that the emulsions wet the surface less easily than the corresponding solutions of the accessory substances. When the aqueous phase wets poorly—for example, in the absence of accessory substances or with soap solutions—the droplets grow in size upon the surface during spraying until under the influence of gravity, they begin to move downward. Their shape is similar to that

TABLE 2

SURFACE TENSION* OF SOLUTIONS OF THE VARIOUS ACCESSORY SUBSTANCES

(All surfaces were 15 seconds old)

Accessory substance	Concentration	Surface tension, γ
	mg per gal	*dynes per cm*
None	0 (tap water)	74 0
Blood albumin	71	65 0
	142	62 6
	284	59 5
	568	57 5
	852	56 4
Hemoglobin	71	70 5
	142	67 4
	284	64 4
	568	61 6
	852	53 8
Sodium oleate	71	69 9
	142	66 6
	284	63 5
	568	58 9
	852	49 1

* These data are as read on the du Noüy tensiometer. To reduce them to absolute values of γ, multiply by factor 0.943, calculated by method of Harkins and Jordan (1930).

shown in figure 3, *B, a, h, i,* and *j*. At no time is there a large area of contact between the emulsion and the surface, and consequently a comparatively small portion of the available oil ever reaches the solid. If a drop of such an emulsion is allowed to move down the surface of a waxed slide or bottle, the oil which separates upon the solid can be seen as isolated spots. Complete coverage can be secured only by allowing many such drops to roll down a given portion of the surface.

When the aqueous phase has moderately good wetting power—for example, with the protein solutions containing 35 to 142 mg per gallon—the drops of emulsion elongate as they move and form long tails in which the oil droplets congregate. Opportunity is thus afforded for oil to deposit upon the surface if it is able, first, to make real contact, and second, to

displace the aqueous phase. As far as these actions occur, the surface changes from the original solid wax to liquid oil. An oil surface is wet by protein solutions much less readily than is wax. For this reason, the emulsions have more difficulty in remaining extended upon the surface as the latter is progressively converted to oil. At some characteristic concentration for each protein, conditions will be such that maximum oil will reach the sprayed surface. With higher concentrations, the aqueous phase has so much wetting power that oil has excessive difficulty in displacing it from the surface; hence, less oil may be expected to be deposited.

Additional information concerning the phenomena which occur during the application of emulsions to wax surfaces was secured by a study of the amounts of the various components deposited during the process of spraying. The total was determined by allowing the tared bottles to drain in the humidity chamber for 5 minutes before weighing, just as in the determination of total deposit of the solutions. The results are shown in figure 6. In this same figure are shown the amounts of oil deposited per bottle from 20 cc runoff of each emulsion. The difference between total deposit of emulsion and oil deposit is the amount of aqueous phase left upon the surface. Obviously the curves representing the deposits of the aqueous phase are in general similar in shape and close to those for total deposit.

As was mentioned above, the photographs of the spraying process show that the various emulsions wet the surface more and more poorly as the deposit of oil increases. The deposits of the entire emulsions and of the aqueous phases bear out this observation.

Thus the decided increase in total deposit with higher concentrations of blood albumin is consistent with the abrupt decrease in the amount of oil deposited, for the surface is thereby wetted more easily. At the same time, the formation of a thin film of the aqueous phase is not possible, so that no decrease in deposit occurs from that cause as occurred with the aqueous solutions alone (fig. 5).

In the case of hemoglobin, the increase in total deposit with small amounts of the protein is caused by increased ease of wetting even in the presence of the oil; but thereafter the uniformly large amount of oil on the surface causes the deposit of emulsion to remain nearly constant as the concentration of hemoglobin increases.

The nearly constant small amount of emulsion deposited in the case of sodium oleate may be explained as a consequence of the very poor wetting properties of this material over the concentration used, together with the fairly constant deposit of oil.

A comparison of figures 5 and 6 will show that with the higher concentrations of accessory substances, more of the aqueous phase containing the proteins remains upon the surface than when only the solutions are used, whereas the reverse is the case with soap. This shows clearly that

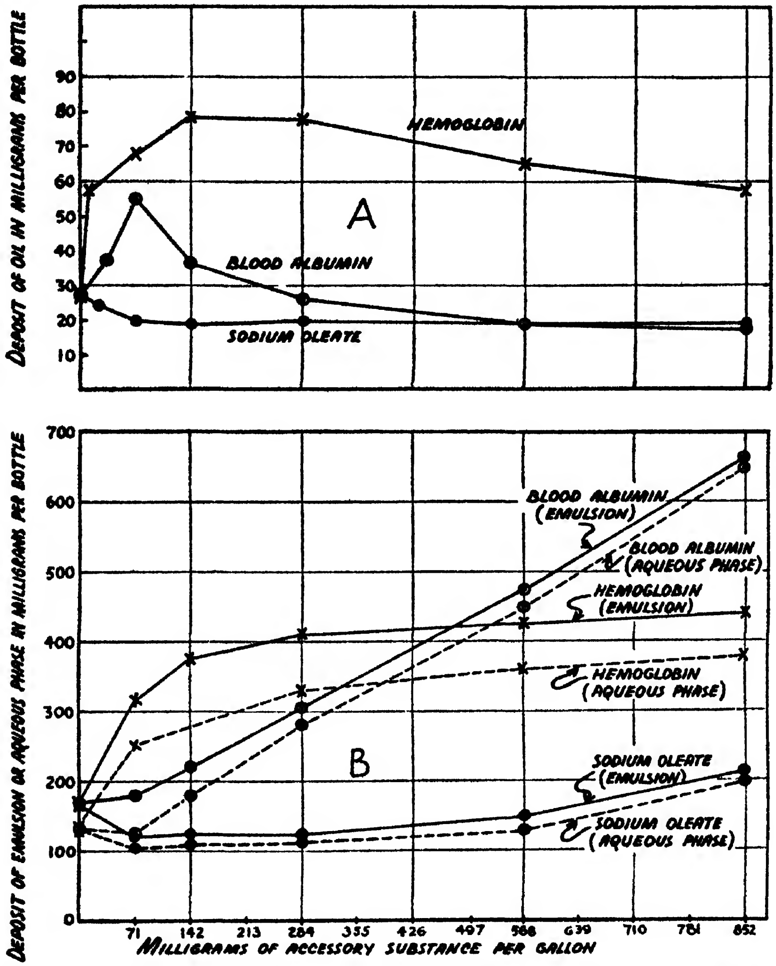

Fig. 6.—Deposit of oil, total emulsion, and aqueous phase resulting from different concentrations of accessory substances.

the greater difficulty in wetting an oil surface and consequent formation of drops instead of sheets increases the amount of the aqueous phase which adheres to the surface. Since the soap solution is not able to form continuous sheets on either wax or oil, a similar reversal would not be expected.

Further information regarding the behavior of oil emulsions during

spraying was obtained by analyses to determine the amounts of accessory substance left upon the wax surface during the process of spraying. Comparison of the data in columns 5 and 2 of table 1, shows that hemoglobin was present in amounts somewhat over half as great as when no oil was in the spray. Thus less accessory substance was deposited from the emulsion than from the aqueous solution; but since drops of the emulsion clung to the oily surface, this experiment shed no light on whether the hemoglobin was still upon the wax, that is, beneath the oil, or whether it was all in the aqueous phase. In order to determine this point, two kinds of experiments were performed.

In the first experiment, upon bottles which had been sprayed with an emulsion containing hemoglobin, the locations of the adherent drops of the whole emulsion were marked, and the spray was then allowed to dry. The spot-test reagent of Lucas (1935) was then applied to various regions of the surface. This reagent is a solution of sodium perborate and benzidine in glacial acetic acid, and it changes from pale straw color to blue in the presence of extremely small amounts of hemoglobin. The results were very clear-cut; for even the smallest drop of the whole emulsion gave an unmistakable response, but the rest of the surface gave no color. It is scarcely possible that the oil covered over any hemoglobin and prevented a reaction; for after standing several hours, as in some of these experiments, the oil enters into the wax layer and leaves the surface exposed again. Furthermore, when a drop of hemoglobin was allowed to dry upon the wax surface and a thin film of oil was added and allowed to stand for a time, the test was very positive. This experiment indicates that hemoglobin follows the aqueous phase and does not remain behind when water is displaced by oil.

The second test of this theory consisted of placing drops of oil and of hemoglobin solution adjacent to one another upon a waxed slide. The areas in which the solution was replaced by oil gave no test for hemoglobin.

No direct study of the behavior of blood albumin or of sodium oleate could be made since similar simple tests are not available. There is every reason to believe that blood albumin acts like hemoglobin. In fact, during the replacement of their solutions by oil, both proteins may be observed to pile up in front of the oil as it advances. Probably not even a unimolecular layer of the water-soluble proteins is left upon wax as the latter becomes covered by hydrocarbon oils. A definite conclusion can scarcely be drawn for soap, which has much more affinity for oil. However, the very poor wetting power of soap solutions for wax is an indication that soap also will follow the aqueous phase.

A number of experiments were made to study the displacing power of oil for the various solutions of accessory substances placed upon waxed plates. In the case of water and of any of the soap solutions within the concentration range used in the spraying work, displacement was complete. The oil crept around and beneath the aqueous drop, which eventually floated upon the oil in the form of a flattened sphere. Probably because water is heavier than oil, the latter was present beneath the drop of water only in a very thin but continuous film.

When a protein solution was used, the initial rate of displacement was slower, and equilibrium was reached in some one of the positions indicated in figure 1. The higher the protein concentration the less the replacement and the more closely the equilibrium state resembled figure 1, II. On account of its great importance in connection with all oil emulsions, the replacement of one phase by the other merits further detailed study.

DISCUSSION

In spite of the importance of the quantity of liquid left upon a plant or other sprayed surface when an aqueous solution of an insecticide or fungicide is applied, very little information on the subject has been recorded. Emphasis is usually laid upon complete wetting of the surface; and of course the importance of reaching each part, at least during the application of the spray, cannot be denied. Unfortunately, however, as shown by Evans and Martin (1935) and in the present investigation, the state of complete wetting is also that of low retention of liquid. The discovery that dissolved proteins become concentrated in the adhering liquid suggests that toxic materials, for example, nicotine or polysulfide, may be held in a similar manner. If this should prove to be the case, then wetting agents would probably affect such behavior, and the possible helpful or harmful effects in this respect of various accessory substances would offer a fruitful field of investigation.

Difficulties in Experimental Work.—Efforts to gain information about the behavior of sprays during actual use must be made under two kinds of handicap. Theory has been developed extensively for static but not for dynamic conditions. At least two additional forces must be introduced into the expressions for the behavior of a liquid sprayed against a solid, that is, gravity and impact pressure. The steady increase in the pressure under which sprays are being applied as more powerful pumps are developed causes the latter factor to be of more and more importance. Until such theoretical advances are made, recourse must be had almost entirely to empirical methods. Here the second difficulty arises; for the necessary technique has been developed but slightly. The complete failure of the

drop method for measuring dynamic angles of contact was pointed out by Ben-Amotz and Hoskins (1937) and illustrated by photographs. The rotating cylinder method of Ablett (1923) is undoubtedly an improvement and merits trial with spray solutions. The ordinary method of changing the angle of incidence of a slide introduced into the liquid has been shown by Evans and Martin (1935) to give results suitable for correlation with deposit of solution formed by their special method of spraying only up to runoff, but it does not apply to the method of overspraying ordinarily used.

Importance of Dynamic Retreating Angle and Static Advancing Angle.—A distinction must be drawn between the situations in which the advancing angle and those in which the retreating angle is of prime importance. When the degree of wetting achieved by a liquid over the surface sprayed and the amount of the liquid retained are the chief considerations, as in the present work, the retreating angle is a controlling factor. Contact having been made with the surface by spraying under pressure, the behavior of the liquid thereafter depends upon its tendency to draw back and roll off. Contact brought about in this way may not be wetting at all in the sense of a meeting of molecules which have a marked degree of affinity for each other.

If the spray is a pure liquid or a solution, that is, consists of one phase only, the amount which will remain upon the solid will not be influenced seriously by the manner in which excess liquid leaves the surface during spraying, but it will be affected greatly by the behavior of that which is present just as spraying is stopped. For this situation, the best criterion is the retreating angle of contact measured under dynamic conditions simulating those prevailing at the time concerned. The migration of solute molecules to an interface and their orientation there are functions of time. Hence a measurement made after more or less prolonged contact, as is the usual method, does not represent the behavior of a spray in ordinary use; for drainage under the influence of gravity occurs rapidly.

The spreading of a spray beyond the limits to which it is driven during application may be of great importance, for example, in reaching beneath the covering of a scale insect or between the petals of an unfolding bud. Such extensions of a liquid usually occur rather slowly. Hence measurements of the advancing angle of contact made under static conditions may be entirely appropriate for study of such behavior.

In short, the static advancing angle is a valuable measure of the ability of a liquid to spread, and the dynamic retreating angle is an equally valuable criterion of its ability to wet. In each case the indication is valid only for the solid upon which the measurement was made, though spread-

ing is more sensitive to surface conditions than is wetting (Bartell and Wooley, 1933). The rapidity with which a liquid changes in shape or position is, of course, dependent also upon its viscosity (Woodman, 1924; Research Staff of General Electric Company, 1922).

Effect of Stability of Emulsion.—The foregoing discussion of the behavior of liquids applies to any single-phase spray, though, in the present work, interest was centered in the aqueous phase largely because it is the carrier for the emulsified oil. The introduction of a second, finely divided liquid, such as oil, broadens the range of variation in the system by at least three additional factors: stability of the emulsion, competition for space upon the solid, and alteration of the surface as oil increases in amount upon it. Of these, the first was discussed by Ben-Amotz and Hoskins (1937), who showed that the more stable an emulsion, other things remaining equal, the less oil will be deposited from a given volume of it. As the stability increases, the individual oil droplets make contact with the solid surface with greater and greater difficulty and fewer of them escape from the layer of emulsifying agent which has collected in the oil-water interface. The ideal method of measuring stability in this case would evaluate this ability of oil to escape from the emulsion to the solid. Such a method, separated completely from wetting phenomena of the aqueous phase, does not seem to have been developed.

The arguments advanced earlier regarding the importance of the dynamic retreating angle of contact as a factor which determines the amount of a homogeneous liquid which will remain upon a sprayed surface must be emphasized from yet another point of view in the case of emulsions. When the aqueous phase has moderately good wetting power, the drops elongate as they roll and in the tails the droplets of oil congregate. They are thereby brought close to the solid, and their chances of making contact with it are enhanced. As emphasized before, at some certain concentration of each accessory substance which allows sufficient wetting, maximum oil will be left upon the surface. This condition is realized with the two proteins but not with the soap; for solutions of the latter do not have pronounced wetting power except at concentrations so high that the deposit, because of the great stability of the emulsion, is extremely low. How much the conditions of replacement of the aqueous phase by oil are influenced by the pressure with which the spray liquid strikes the surface apparently has not been investigated. Until this information is available, experiments on replacement under static conditions cannot be used for quantitative comparison of various emulsions, but they certainly permit qualitative comparison.

Primary and Secondary Deposit.—The gradual alteration in the

sprayed surface from the original solid through spotted attachment and spreading of oil droplets to the final continuous layer of oil affords opportunity for division of the process into steps. The terms "primary" and "secondary" deposit were used by Smith (1933) and with slightly modified meaning by Hensill and Hoskins (1935). In the light of certain ideas developed in this report, they may be further elaborated. The primary deposit really consists of two parts. In the beginning, oil droplets are

TABLE 3

FRACTIONAL PART OF TOTAL OIL DEPOSIT CONTRIBUTED BY SECONDARY DEPOSIT

Concentration of accessory substance	Weight of aqueous phase left			Minimum weight of oil corresponding to aqueous phase			Total oil deposit (from fig. 7)			Minimum fraction of total oil deposit from secondary deposit		
	Blood albumin	Hemoglobin	Sodium oleate	Blood albumin	Hemoglobin	Sodium oleate	Blood albumin	Hemoglobin	Sodium oleate	Blood albumin	Hemoglobin	Sodium oleate
mg per gal.	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*per cent*	*per cent*	*per cent*
0	140	140	140	2.4	2.4	2.4	28.0	28.0	28.0	9	9	9
71	125	250	155	2.1	4.2	2.6	55.0	68.0	20.0	4	6	13
142	180	300	165	3.1	5.1	2.8	37.0	78.5	19.5	8	6	14
284	280	330	175	4.8	5.6	3.0	26.0	78.0	20.0	18	7	15
568	450	360	205	7.7	6.1	3.5	19.0	65.0	19.0	41	9	18
852	650	380	300	11.0	6.5	5.1	17.5	57.5	19.0	63	11	27

placed upon wax and in almost innumerable points a thin coating is developed as the oil spreads. This may be called the first stage, during which the wetting power of the aqueous phase for wax determines the manner and extent of contact between spray and solid. Displacement of the aqueous phase by oil corresponds in general to that found when a drop of oil and a drop of solution of the accessory substance are placed adjacent to one another upon a waxed slide. As the surface becomes changed from wax to oil, the first stage merges into the second, in which the wetting power of the aqueous phase for a surface of oil determines the degree of contact. Displacement of the aqueous phase by additional oil from the spray is very rapid and complete. Hence the major portion of the primary deposit is laid down during this period.

Secondary deposit is that part of the total oil which reaches the surface from adhering drops or sheets of emulsion when the water in the latter evaporates. The data needed for determination of its magnitude are given in figure 6, *if the assumption is made that the aqueous phase which is left upon the bottles at the conclusion of spraying has the same composition as the original emulsion.* The results are given in table 3.

With low concentrations of the accessory substances, only a small portion of the total oil is contributed by the secondary deposit; but at higher concentrations, this is increasingly important, particularly in the case of blood albumin.

There is evidence from visual observation that the assumption made in these calculations is not valid in all cases; for the residue of emulsion resembles the product formed by creaming, particularly when the larger amounts of accessory substances are present. That is, under the circumstances in which the drops elongate as they roll down the surface, the emulsion becomes enriched and more oil is left as a secondary deposit than is calculated from the weight of emulsion upon the surface. Hence the data of table 3 on secondary deposit should be taken as minimal values.

In general, high secondary deposit is undesirable, for such oil will be left in very localized regions and can contribute to the general deposit only by spreading upon the surface. While this may occur to some extent with oil, it is impossible in the case of a suspended solid such as bordeaux mixture or lead arsenate. Consequently the secondary deposit of such substances is of little value, except with such pests as are attracted to spotty deposits of toxic material—for example, the walnut husk fly, *Rhagoletis completa* Cresson.[6]

The nature of the curves relating total deposit with concentration of accessory substance can be qualitatively explained for blood albumin, hemoglobin, and sodium oleate in terms of the wetting power of the aqueous phase, ease of replacement of the aqueous phase by oil, and stability of the emulsions. The characteristic differences between typical wetting agents and typical emulsifiers are consistent with their relative abilities to deposit oil. The above properties, however, are complex in nature and give no final answer to the problem.

The information gathered from these experiments indicates the possibility of formulating a theory in terms of three fairly simple properties of an oil emulsion:

1. The retreating angle of contact of the aqueous phase upon the chosen solid. This must be determined under dynamic conditions, and at present high-speed photography seems to offer the best approach.

2. Ease of escape of oil from the minute droplets of the emulsion onto the surface. The difficulty in testing this property is separation of it from wetting power of the aqueous phase.

3. Rate of replacement of the aqueous phase by the oil. Theoretically this is involved in equation 2, $\gamma_{SB} = \gamma_{SA} + \gamma_{AB} \cdot \cos \theta_{AB}$. Direct measure-

[6] Personal communication from Dr. A. M. Boyce.

ment of θ_{AB}, which may be called the angle of replacement, is very difficult under dynamic conditions, and the displacement method of Bartell and Osterhof (1927) and Bartell and Whitney (1932) is complicated in the present case by the solubility of oil in the beeswax.

Wetting Agents as Spreaders of Oil.—The question of the rôle of water-soluble wetting agents in promoting the spread of oil has been discussed by certain previous workers (de Ong, Knight, and Chamberlain, 1927; Smith, 1933; Knight and Cleveland, 1934). Smith, who used the term "spreader" in the same sense in which "accessory substance" is used in this report, said: "It appears that one function of a spreader is to cause the oil to spread on surfaces upon which it will not spread otherwise" and "As the water evaporates, the spreader lays down a coating over the surface over which the oil spreads in a uniform film."

The truth of the second statement is substantiated by the experimental observation that oil spreads over a region in which a protein solution has dried on beeswax several times as fast as upon the untreated beeswax. The protein, however, must be dry. Hence the effect can occur with oil emulsions only after the water has evaporated. The evidence from the replacement experiments indicates that water-soluble accessory materials promote wetting by the aqueous phase only. But there are differences in the tendencies of proteins and of soaps to collect in the various interfaces. Thus, sodium oleate has a strong affinity for the oil-water interface and forms very stable emulsions, which wet wax but poorly because the soap has less affinity for the wax-water interface. Blood albumin and hemoglobin promote wetting by water very strongly but emulsify oil poorly. Possibly an accessory substance somewhat soluble in both water and oil would be retained sufficiently by the oil to promote the spread of the oil upon wax during the time of spraying, but this would necessitate the presence in the molecule of three groups having affinities for water, oil, and wax, respectively.

SUMMARY

The application of a spray is a dynamic process, and hence attempts to relate the deposit of oil obtained from various emulsions should be based upon measurements made under conditions approximating those of use. With a standard surface of beeswax as the solid sprayed and a standard method of spraying, a study has been made of the relations between concentration of blood albumin, hemoglobin, or sodium oleate and the following properties: amount of the aqueous solution and of the accessory substance deposited in the absence of oil; deposit of oil, of aqueous phase, of accessory substance, and of all components when emulsions were used;

ease of wetting of wax by solutions of the accessory substances and by the entire emulsion; replacement of aqueous phase by oil; and stability of the emulsions.

The amount of oil deposit can be explained at least qualitatively as follows: When only oil and water are present, the emulsion wets poorly, and drops roll on the surface with minimum area of contact. Addition of a protein promotes wetting and opportunity for oil to reach the surface. Hence deposit is increased until formation of large sheets of the aqueous phase upon the surface and resistance to displacement of the aqueous phase by oil lead to a decrease in oil deposit with higher concentration of protein. Soap promotes wetting so little that a corresponding increase in oil deposit does not occur. With all three accessory substances, the increase in stability of the emulsions diminishes oil deposit. Water-soluble substances follow the aqueous phase and increase spreading of oil only after the water has evaporated.

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THE USE OF SELENIUM IN SPRAYS FOR THE CONTROL OF MITES ON CITRUS AND GRAPES

W. M. HOSKINS, A. M. BOYCE, AND J. F. LAMIMAN

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THE USE OF SELENIUM IN SPRAYS FOR THE CONTROL OF MITES ON CITRUS AND GRAPES[1]

W. M. HOSKINS,[2] A. M. BOYCE,[3] AND J. F. LAMIMAN[4]

INTRODUCTION

For several years it has been increasingly obvious that the standard methods used for control of several plant-infesting mites in California are unsatisfactory (Boyce, 1936).[5] For this reason the California Agricultural Experiment Station projects on the citrus red mite, *Paratetranychus citri* McG., and on the Pacific red spider, *Tetranychus pacificus* McG., have been carried on very actively. One feature of such projects is the examination of new materials under both small-scale and practical conditions. In pursuance of this work, a proprietary selenium-containing preparation called Selocide was tested by Lamiman (1933) against the Pacific red spider on grapes. This material had been used successfully by Gnadinger (1933) for control of the common red spider, *Tetranychus telarius* (Linn.), in greenhouses. The first small-scale field trials on both grapes and citrus were of so much promise that a new project[6] was organized in 1934 for the purpose of making a thorough study of the practical utility of Selocide alone and in combination with other substances for the control of mites affecting citrus and grapes.

It is an interesting coincidence that at about the time when the use of selenium for plant protection was suggested, attention was first directed to this element as a harmful ingredient of animal and human foodstuffs. Since the first settlers went into the semiarid Great Plains region of the United States, a livestock ailment of unknown origin has been noticed in many areas of the present states of South Dakota, Nebraska, Kansas, Wyoming, and other neighboring states. The disorder assumed, in general, either an acute or a chronic form, which became known as "blind

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[5] See "Literature Cited" for further data on citations, which are referred to in the text by author and date of publication.

[6] A special committee was appointed by the Dean of the College of Agriculture to conduct the investigation. The members are: W. B. Herms (chairman), D. R. Hoagland, P. L. Kirk, C. D. Leake, and the authors of this report.

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staggers" and "alkali disease," respectively. The first written account seems to have been made by Madison (1860) and concerns an outbreak among cavalry horses at Fort Randall, Territory of Nebraska, the site of which is now included in South Dakota. Madison attributed the trouble to grazing of the affected animals in a certain ravine. Since that time, occasional references to the disease have been made in agricultural experiment station reports, but little advance was made in determining the cause. Among practical stockmen, it was attributed usually to alkaline drinking water.

In 1929 Franke of the South Dakota Agricultural Experiment Station began the first comprehensive investigation of alkali disease and soon proved (Franke, Rice, *et al.*, 1934) that it invariably followed the ingestion of grains from certain districts in the state. The discovery by Robinson (1933) that selenium was present to the extent of 10–12 p.p.m. in a sample of the so-called "lethal wheat" at once gave a new direction to the investigation and led to extensive feeding trials with both inorganic selenium compounds and seleniferous plants in many laboratories. A warning against hasty adoption of selenium-containing materials as sprays was issued by Nelson, Hurd-Karrer, and Robinson (1933).

In the light of these conditions, the investigation of the California Agricultural Experiment Station was designed to include, in addition to a study of the effectiveness of the selenium preparation for control of mites, determination of the amounts of selenium residue upon fruit as the result of various spray programs, of the accumulation of selenium in the soil beneath sprayed plants, and of the absorption of this selenium from the soil. Also, preliminary plant-rearing experiments and feeding trials of inorganic selenium and seleniferous plant materials were made.

This report includes accounts of the experimental procedures and results, as well as a summary of published information on the general occurrence and toxicity of selenium and a discussion of its probable health hazard when used in the recommended manner upon citrus and grapes.

CHEMICAL PROPERTIES OF SELENIUM AND ITS COMPOUNDS

Selenium (Se) has an atomic weight of approximately 79 and lies between sulfur and tellurium in the sixth group of the periodic table of the elements. Its closest neighbors in the adjacent groups are arsenic and bromine. In the uncombined state, selenium occurs in several allotropic forms, of which the more common are the grayish-black crystalline, the black amorphous, and the red amorphous. The latter is formed when selenium is reduced from one of its compounds, is the most reactive form

of elementary selenium, and under many conditions is converted to the black crystalline state. The chemical properties of selenium are similar to those of sulfur. Both vary in valence from —2 to + 6. Typical compounds representing the various valences are: —2, hydrogen selenide, H_2Se; + 2, selenium sulfide, SeS; + 4, selenium dioxide, SeO_2, which unites with water to form selenious acid, H_2SeO_3; + 6, selenium trioxide, SeO_3, which is known only in the hydrated form as selenic acid, H_2SeO_4. The two oxides and their corresponding acids and salts are strikingly different in behavior: the tetravalent state is readily formed when nearly any ordinary oxidizing agent acts upon free selenium or its lower compounds, but only the most powerful oxidizing agents—for example, permanganate—can raise it to the hexavalent state. However, when fully oxidized, selenium can be reduced only with difficulty. Hence the selenate compounds are usually comparatively inert. On the contrary, the selenite state is reactive and may be readily reduced to a lower valence.

Despite its close chemical relationship to sulfur, selenium until recently aroused little interest as a material for plant protection. The work of Lougee and Hopkins (1925) proved the toxicity of several selenium compounds to certain fungi, but damage to sprayed trees was severe.

A 30 per cent solution of a mixture of potassium hydroxide, ammonium hydroxide, sulfur, and selenium in the proportions corresponding to the empirical formula $(K\,NH_4\,S)_5\,Se$ is the proprietary product called "Selocide." It contains 48 grams of selenium per liter, or approximately 6.4 ounces of selenium per gallon. Dilutions of 1:100 and 1:1,000 give sprays containing 0.0480 and 0.0048 per cent selenium respectively, and other dilutions are of proportional concentrations. The first work done for control of the common red spider was usually at a dilution of 1:200 (Gnadinger, 1933) but Lamiman (1933) found much more dilute solutions—for example, 1:600 and 1:800—to be effective outdoors against the Pacific red spider on grapes, and these same concentrations have been used widely on citrus. For proper wetting of most leaf and fruit surfaces, a wetting agent, such as a soluble protein, is advisable.

The reactions which occur when a concentrated solution of Selocide is diluted with water, as in preparing a 1:800 spray mixture, do not appear to be known. Observation shows, however, that a large part, if not all, of the selenium is liberated as very finely divided, dark-red particles, which appear to be the red allotropic form of the element. When finely ground sulfur is added to Selocide, the residue has a persistent effect which is shown by neither component alone (see p. 119). There is no chemical evidence that a compound is formed, but the effect may possibly be due to some new product, such as selenium sulfide.

SELOCIDE ON CITRUS AND WALNUTS

The Citrus Red Mite.—The proprietary selenium preparation Selocide has been rather extensively studied in connection with the control of the citrus red mite (spider), *Paratetranychus citri* McG., in southern California during the period from 1933 to 1937. This mite is one of the major pests of citrus in many areas (Boyce, 1936). The total acreage of citrus affected probably exceeds 125,000 acres.

Until the advent in about 1925 of the highly refined petroleum oils for citrus sprays, sulfur in some form had been largely used for the control of the citrus red mite. During the past ten years, however, the use of sulfur or its compounds for the control of this mite has been very limited. This fact is due principally to the following causes: (1) superiority of oils for this purpose and the more general use of oils as a combination treatment for control of scale insects and this mite; (2) the unsatisfactory results in the control of this mite by sulfur as compared with the results secured in former years; and (3) hazard of fruit injury caused either by dry, hot winds in certain sections or by a sudden increase in daily temperature after sulfur applications. Thus, at present, petroleum oil is the principal material used for the control of the citrus red mite.

In many sections, under ordinary conditions, one application of oil spray in the summer or fall affords satisfactory protection from injury by this mite for about one year. However, after the use of an oil spray, if climatic conditions are extremely favorable to mite development throughout the winter and spring or throughout the summer, there usually develops a need for mite control with a material that contains little or no petroleum oil. In the coastal areas, there is commonly a necessity for citrus-red-mite control in the spring or early summer, even though oil spray was applied the preceding summer or fall. From the standpoint of adverse tree reaction, more than one treatment of oil spray at full dosage on citrus during a year is generally considered inadvisable. A large number of growers prefer to abstain from the use of oil altogether and therefore employ HCN fumigation in the control of scale insects. Fumigation usually has no appreciable effect in citrus-red-mite control; in fact, the mite populations commonly increase more rapidly after fumigation than otherwise, since many of their natural enemies are killed. Evidently there is an urgent demand for a material other than oil for use in the control of the citrus red mite.

Many laboratory tests and several hundred control experiments in the field were conducted in the various citrus-red-mite areas. These were designed principally to determine the most effective (1) concentration of

Selocide; (2) adjuvant, such as lime-sulfur, wettable sulfur, or oil; and (3) spreading and wetting agent. The data show that Selocide at 1:800 is as effective as any of the higher concentrations that are practical to use. An adjuvant is essential for maximum efficiency, the most satisfactory ones apparently being lime-sulfur at 1:300, wettable sulfur at 1 to 2 pounds per 100 gallons of spray mixture, light-medium mineral oil at 1:300, and a combination of the oil and wettable sulfur.

The Selocide-and-lime-sulfur or wettable-sulfur combination affords a high degree of kill of the mites contacted. It kills only a small percentage of the eggs; but a residuum is deposited on the tree that kills the young mites shortly after they are hatched, even during relatively low temperatures and under conditions when the residuum from a 2 per cent lime-sulfur spray or regular sulfur dust is ineffective. Definite information regarding the chemical nature of this residuum is not available. The use of either of these Selocide-sulfur combinations may not effect satisfactory control when rains follow the application within a period of several weeks and wash the residuum off the foliage and fruit, for under these conditions the mites that subsequently hatch survive.

The Selocide-oil combination effects a high initial kill of the mites and eggs contacted; however, there is apparently no residual effect from the use of this combination, and, therefore, unless the application is very thorough, the results may not be satisfactory. A comparison of many parallel field experiments with the Selocide–lime-sulfur and Selocide–wettable-sulfur combinations versus the Selocide-oil combination shows that the last-named has generally afforded a higher degree of control, although in certain experiments the two sulfur combinations were superior.

When it became evident that each combination possessed certain specific merits, tests were conducted in which both lime-sulfur and oil, each at 1:300, and both wettable sulfur at 1 pound per 100 gallons and oil at 1:300, were combined with Selocide. Tree injury occurred under certain conditions with the Selocide–lime-sulfur–oil combination. However, the Selocide–wettable-sulfur–oil combination appears to be noninjurious to the tree and has afforded very effective control of the mite. A fairly large acreage has been treated commercially with this combination with satisfactory results.

Several spreading and wetting agents, such as sodium caseinate, calcium caseinate, blood albumin, soaps, sulfonated plant oils, and sulfated higher alcohols have been used with Selocide in the several combinations. According to present information, sodium caseinate is the most practical spreading and wetting agent for use with Selocide. Blood albumin should

not be used on citrus in any Selocide combination in which oil is included, since extensive tests have shown that serious staining or spotting of the fruit may occur from this combination of materials.

In summing up the field studies with Selocide on citrus, it may be said that this material, used according to the following formula, has given satisfactory control of the citrus red mite and is the one suggested for use where Selocide is considered:

Selocide	1 pint
Wettable sulfur	1 pound
Light-medium oil[7]	⅛ gallon
Sodium caseinate	3 to 4 ounces
Water	100 gallons

The regular dusting sulfur may be used in the same amount and rendered wettable by mixing the caseinate with the dry sulfur in a large bucket and then adding water under pressure with the spray gun until the sulfur is suspended in the water.

With regard to the choice of oil, the straight oil, as used in tank-mix, appears to have certain advantages over paste emulsions or emulsive oils, since both of the latter types of oils contain wetting and spreading agents which, when included with the caseinate regularly used with Selocide, may possibly cause excessive runoff of the spray mixture. In commercial practice, however, the paste emulsions and emulsive oils are used with success.

The order in which the several materials are put into the spray tank is of importance. The sulfur-casein, prepared as previously indicated, should be put into the tank first, when the water is about level with the agitator shaft. The Selocide should be added next when the tank is about one-third filled, and the oil added last when the tank is about two-thirds filled. If paste emulsion or emulsive oil is used, the recommendations of the manufacturer should be followed with regard to when the oil is to be added; however, in all instances the sulfur should be put into the tank before the Selocide.

Other Mites and Insects on Citrus.—The Selocide-sulfur-oil combination has afforded satisfactory control of the six-spotted mite, *Tetranychus sexmaculatus* Riley, on citrus. It has not been entirely satisfactory, however, in the control of the silver or rust mite, *Phyllocoptes oleivorus* (Ashm.), which is of importance only sporadically in limited areas in San Diego County. Since this mite is readily and inexpensively controlled through applications of sulfur applied as a dust, Selocide would not be considered in this connection unless the citrus red mite or the six-

[7] Unsulfonatable residue 92 per cent; distillation range: 10 per cent at 587° F, 50 per cent at 632° F, 90 per cent at 702° F.

spotted mite or both species were also present. Under such conditions, the silver mite could probably be satisfactorily controlled by increasing the amount of wettable sulfur from 1 pound to 4 or 6 pounds per 100 gallons in the Selocide-sulfur-oil combination.

Selocide as used, either experimentally or commercially, has not been shown to possess measurable value in the control of any of the *insect* pests of citrus with the possible exception of the citrus thrips, *Scirtothrips citri* (Moult.). In this particular instance, those thrips that are contacted by the Selocide spray mixture are killed; and when either lime-sulfur or wettable sulfur is included in the spray mixture, the residuum is effective in destroying the newly hatched nymphs for a period of about 1 week following the treatment. The Selocide-sulfur-oil combination would probably be of considerable value for the combined treatment of citrus red mite and the citrus thrips under certain conditions if the amount of wettable sulfur were increased from 1 pound to 4 or 6 pounds per 100 gallons, as recommended for the silver mite.

Compatibility of Selocide with Other Materials Commonly Used in Sprays on Citrus.—Selocide may be used with nicotine sulfate for the combined control of the citrus aphids, *Aphis spiraecola* Patch, *Aphis gossypii* Glover, and *Toxoptera aurantii* (B. d. Fonse) and several species of mites previously mentioned.

Where zinc compounds are used for the correction of mottle-leaf or little-leaf (Parker, 1938) of citrus trees, either zinc oxide or zinc sulfide may be satisfactorily combined with Selocide in the spray mixture. However, the zinc-sulfate–hydrated-lime mixture is incompatible with Selocide.

Limitations to the Use of Selocide on Citrus.—The use of Selocide on Valencia oranges in the coastal sections of Los Angeles County and the northern fringe of Orange County is attended with considerable danger of injury to the fruit during the period from late winter until the mature fruits are harvested. In certain seasons in this particular area, a rind weakness develops which is apparently due to environmental conditions. When sulfur is applied, it commonly accentuates this condition, with the result that one or more spots may develop on a fruit. Affected fruits either drop before harvest or otherwise are marketed in the lower grades. The sulfur or other constituents of Selocide contribute to the incidence of this type of fruit spot when other environmental conditions are favorable.

In the Chula Vista area of San Diego County, the peel of lemons not uncommonly becomes stained in small areas, or spotted, after use of Selocide. Affected fruits do not color uniformly; and while their quality is

apparently not impaired otherwise, they are not marketable in the higher grades for this reason. In this particular area, the cause of the stain on the peel of the fruit appears to be related to the occurrence of relatively large amounts of certain salts commonly present in the water that is used for spraying.

A type of stain on the peel of Navel oranges similar to that described above on lemons occasionally results from the application of Selocide. In the instances observed, however, the treatment was applied at the season when the fruit was "breaking color." Through further studies, the causes of the stain on the peel of lemons and oranges may perhaps be determined, and the difficulty may be prevented.

When excessively high temperature conditions prevail within about 10 days after Selocide treatment, some of the fruit that is exposed to the direct sunlight is commonly injured. The injury is typical of that due to high temperatures after the application of sulfur.

Effect of Selocide on the General Vigor and Productiveness of Citrus Trees.—Thousands of acres of oranges and lemons have been treated with Selocide during the past five years at different seasons of the year and under widely varying conditions. A relatively large acreage has received as many as five treatments and a smaller acreage as many as seven or more treatments during that period. In no instance has there been any observable adverse effect upon the general vigor or productivness of such treated trees, nor has there been observed any impairment in the texture or quality of the fruit other than the features previously mentioned.

Studies with Selocide on Persian Walnuts.—Selocide has been fairly extensively used experimentally and commercially on Persian walnuts in the control of the common red spider. One treatment annually of the material used according to the formula previously given for citrus has afforded entirely satisfactory control. While treatment for this mite is necessarily applied in midsummer in the hot interior valley areas, no evidence of injury due to the material has been observed.

Effect of Selocide on Spraymen.—Many spraymen have applied large volumes of Selocide spray mixture to citrus over relatively long continuous periods during several years without any observed deleterious effects to date upon their health and with no more personal discomfort than would be experienced through the application of light dosages of lime-sulfur spray. More than 40,000,000 gallons of Selocide spray have been applied in the United States, much of it indoors in greenhouses. Gnadinger (1937) is authority for the statement: "No workman engaged in the manufacture or packing of Selocide has experienced any illness or injury traceable to Selocide or remotely resembling selenium poisoning."

SELOCIDE AS A CONTROL FOR THE PACIFIC RED SPIDER ON GRAPES

The Pacific red spider, *Tetranychus pacificus* McG., which has become a serious pest of grapes in the grape-growing areas of northern California, was first observed as a grape pest in 1928 in the San Joaquin Valley.

The habits of this mite are such that a satisfactory control is very difficult. In late summer and early fall, the adult females hibernate beneath the bark of the grapevines, where they may be found in groups of a few to several hundred. As many as 2,000 have been taken from the bark of a single vine, with a survival of 50 to 80 per cent the following spring. These overwintering females emerge in March and feed on the opening buds or may be found on various weeds, such as filaree, sheperd's-purse, and malva. The appearance of the mites is influenced by weather conditions and during a cool spring may be delayed for two to three weeks; thus the maximum infestation may be delayed until late summer.

Each female deposits from 50 to 100 eggs over a period of 2 weeks to a month, and only 10 to 14 days are required for hatching and development of the mite under summer conditions, with the result that several generations may be produced in a single season. The greatest populations occur in late July or August, when infestations ranging from 600 to 1,400 mites per leaf have been observed on leaves showing severe mite injury. The rapidity with which these mites may multiply is indicated by the increase in the average number of Pacific red spider per leaf from 18.8 on June 12 to 343.7 on August 12, with a maximum of 1,458 on untreated vines; and from 0.2 on June 12 to 192.1 on August 12, with a maximum of 296 on treated vines. A large population of mites in combination with high temperatures and low humidity then produces severe vine injury by defoliation and reduces the quality of the crop by exposure to sunburn as well as by lowered sugar content.

Early attempts to control this mite by means of the accepted methods of red-spider control resulted in a number of problems. Until about 1925, when highly refined mineral-oil sprays were developed for summer use, application of sulfur, in its various forms, was the recognized red-spider control. Sulfur dusts are ineffective in controlling the Pacific red spider, even though several applications are made in the same season. Lime-sulfur and sulfur sprays, the chief control before the advent of summer oil sprays, were unsatisfactory due to the excessive injury to new growth in early summer and to the blotchy residue left on the fruit by later applications. Summer oil sprays, the method most widely used against red spiders on deciduous trees since 1925, gave excellent initial results

but did not prevent reinfestation by the few individuals escaping the treatment. These sprays also removed the bloom from the grapes and produced shiny, soft, flabby berries, and thus reduced the quality and market value of the product. However, oil sprays can be used to reduce the infestation of new growth by the overwintering females before the blossoms appear.

Selocide,[8] a spray material developed by Gnadinger (1933), while conducting experiments on the control of the common red spider, *Tetranychus telarius,* in greenhouses, was used in 1932 (Lamiman, 1933) under field conditions. In these experiments, the Selocide concentrate, at a dilution of 1:800 (1 gallon to 800 gallons of water), with a cocoanut-oil soap spreader, was highly effective under field conditions, whereas a dilution of 1:200 had previously been recommended for greenhouse conditions. As these applications were made in July, the 95–100 per cent control obtained on juice-grape varieties prevented serious reinfestation.

In 1933 the experimental work was extended to include the Thompson Seedless variety, and applications were begun as soon as mite damage appeared in early June. Reinfestation of the vines became apparent after about 4 weeks, even though the initial kill was high; in many cases additional applications were needed to prevent defoliation.

During the same year, Gnadinger (1933) found that the effectiveness of the Selocide spray in the control of the citrus red mite was greatly increased by the addition of lime-sulfur (1:300) or a light medium mineral oil (1:300). Since neither of these combinations could be applied on grapes, wettable sulfur[9] in varying amounts was added to the Selocide spray in 1934 and 1935. Reinfestation was greatly delayed, and where applications were made in late June or early July, the vines retained their foliage and matured the crop, although some mite damage occurred before the mites began to hibernate in August and September.

The effectiveness of the various concentrations of Selocide without sulfur and of other materials is shown in table 1. While a dilution of 1:800 gave satisfactory results under certain conditions, a 1:600 or 1:500 dilution was more effective, especially on the Thompson Seedless variety with its denser foliage. Table 2 shows the results obtained by the addition of varying amounts of sulfur to the different dilutions of the Selocide spray. At the dilutions used, Selocide did not produce any injury to vines or fruit.

The results shown in the tables were obtained by collecting leaves at

[8] The effect of Selocide on spraymen is discussed in the previous section, p. 122.

[9] The wettable sulfur was prepared by adding either blood-albumin spreader or caseinate spreader to ordinary fine dusting sulfur; the combination was then mixed into a paste and added to the spray tank.

random in the treated areas; from each group, 20 leaves were selected at random. On these leaves, five areas (each 5 sq. cm) were marked off with a circular rubber stamp. These areas were arbitrarily selected as being those most likely to be infested by the mites. Comparisons of the count areas with the remainder of the leaf showed that approximately one-

TABLE 1

Comparison between Selocide without Sulfur and Other Materials for the Control of Pacific Red Spider on Grapes

Materials used	Date of application	Average population per leaf based on number in count areas					
		Before treatment	After 3 days	After 10 days	After 30 days	After 50 days	After 70 days
Selocide 1:400+4 oz. blood-albumin spreader* to 100 gals.	June 23	31	2	1	3	39	
	July 7	37	2	6	21	53	
Selocide 1.400+8 oz. Ortho casein and 4 oz. blood-albumin spreader to 100 gals.	July 30	66	32		91		
Selocide 1:600+4 oz. blood-albumin spreader to 100 gals.	June 2	69	13	3	4	20	90
Selocide 1:600+4 oz. blood-albumin spreader to 100 gals.	July 7	40	4	7	33	103	
Selocide 1:600+12 oz. Fluxit† to 100 gals.	June 2	69	6	2	0.7	14	57
Selocide 1:600+8 oz. blood-albumin spreader to 100 gals.	June 20	42	2	3	31		
Selocide 1:600+8 oz. Ortho casein and 4 oz blood-albumin spreader to 100 gals.	July 26	91	11	41	86		
Light-medium tank-mix oil 1½ per cent	June 2	69	1	4	4	30	
Wettable sulfur 6 lbs. to 100 gals.	June 17	43	6	7	55		
	June 17	38	11	15	55		
Wettable sulfur 6½ lbs. to 100 gals.	July 8	37	13	19	47	78	
Lethane§ 420, 1:600	June 19	45	17	30	64		
Lethane 420, 1:300	July 30	27	26	35			
Ortho derris 4 lbs. to 100 gals.	June 19	36	7	20	87		
Untreated	June 5‡	12		33	48	98	
	June 18‡	32		54	71		

* A commercial product containing 1 part powdered blood albumin (about 98 per cent water-soluble) and 3 parts fuller's earth.

† A commercial product consisting chiefly of calcium caseinate plus excess lime.

‡ First counts made.

§ A commercial organic thiocyanate.

third of all the mites present occurred in the count areas, which represented an average of 20 per cent of the leaf surface. This method made possible the examination of a greater number of leaves from each experimental area.

The results of the tests described above may be summarized as follows: The Selocide–wettable-sulfur combination not only affords a high initial kill of the mites contacted, but it also has a lasting effect, for a residuum is apparently deposited which kills the young mites after they have

hatched out and the mites which were in the molting stage at the time of the application. At present, the only satisfactory summer control for the Pacific red spider on grapes is the use of this Selocide–wettable-sulfur combination. However, oil sprays may be used in spring and early summer against the overwintering adults on the new growth before the grapes develop.

TABLE 2

EFFECT OF ADDING VARYING AMOUNTS OF SULFUR TO SELOCIDE IN THE CONTROL OF THE PACIFIC RED SPIDER ON GRAPES

Materials used	Date of application	Average population per leaf based on number in count areas					
		Before treatment	After 3 days	After 10 days	After 30 days	After 50 days	After 70 days
Selocide 1:400+6½ lbs. sulfur and 12 oz. Fluxit† to 100 gals.	July 8	37	0.0	0.0	0.2	0.8	.
Selocide 1:400+2 lbs. sulfur and 6 oz. blood-albumin spreader* to 100 gals.	July 30	57	0.1		0.1		
Selocide 1:500+2 lbs. sulfur and 8 oz. blood-albumin spreader to 100 gals.	July 2	34	2	0.2	9	24	.
Selocide 1:500+2 lbs. sulfur and 6 oz. blood-albumin spreader to 100 gals.	July 14	58	0.6	0.1	3	.	
	July 31	31	0.3	1.0	2		
Selocide 1:600+2½ lbs. sulfur, 8 oz. Fluxit, and 4 oz. blood-albumin spreader to 100 gals.	July 24	33	0.1		3		
Selocide 1:600+6½ lbs. sulfur and 8 oz. Fluxit to 100 gals.	July 11	42	0.2	0.9	2	21	
Selocide 1:600+2 lbs. sulfur and 8 oz. blood-albumin spreader to 100 gals.	June 3	14	1.0	1.0	1.0	29	
	June 20	18	0.1	1.0	1.0	41	85
Selocide 1:600+1 lb. sulfur and 8 oz. blood-albumin spreader to 100 gals.	June 3	11	0.4	2	9	54	
Selocide 1:600+½ lb. sulfur and 8 oz. blood-albumin spreader to 100 gals.	June 3	13	1.0	2	28	64	
Selocide 1:800+6½ lbs. sulfur and 8 oz. Fluxit to 100 gals.	July 11	50	.	1.0	2	18	
Selocide 1:800+5 lbs. sulfur, 4 oz. Fluxit, and 4 oz. blood-albumin spreader to 100 gals.	July 12	54	1.0	3	4	45	
Selocide 1:800+2 lbs. sulfur and 8 oz. blood-albumin spreader to 100 gals.	July 18	13	1.0	1.0	1.0	3	29
Selocide 1:800+1 lb. sulfur and 6 oz. blood-albumin spreader to 100 gals.	July 28	33	0.3	2	3	9	25
Selocide 1:1,000+5 lbs. sulfur, 4 oz. Fluxit, and 4 oz. blood-albumin spreader to 100 gals.	July 12	68	2	4	26	63	

* A commercial product containing 1 part powdered blood albumin (about 98 per cent water-soluble) and 3 parts fuller's earth.

† A commercial product consisting chiefly of calcium caseinate plus excess lime.

SELECTION OF SAMPLES AND METHODS OF ANALYSIS

Collection of Samples.—Fruit and leaves were selected at random, with special care to disturb the surface as little as possible. They were placed in clean bags, packed in boxes, and taken or sent to the laboratory at once.

Selenium adheres well and it is felt that no serious losses occurred during the necessary handling. Four to eight fruits were used for each analysis. Grape samples were composed of portions of several bunches from different vines whenever possible.

Soil samples were taken by members of the Station staff at Riverside. In order to obtain data for extreme conditions, the samples were taken near the edge of the trees where drip of spray would be at a maximum. Separate samples were taken in each case at the depths of 0–6 inches, 6–12 inches, 12–24 inches, and 24–36 inches. In most cases these were analyzed separately.

Review of Methods of Selenium Analysis.—Many methods have been devised for the quantitative analysis for selenium. Those which appear to have been most successful are based upon the following properties of the element: (*a*) Se^{+6} boiled with 3 to 12 *N* HCl is reduced to Se^{+4} (Norris and Fay, 1896, Noyes and Bray, 1907); (*b*) Se^{+4} in the same hot acid solution is reduced to free selenium by SO_2, hydrazine, hydroxylamine, etc.; (*c*) metallic selenium reacts with strong nitric acid to give only selenious acid (Lyons and Shinn, 1902); (*d*) selenious acid reacts with iodide to liberate free iodine, which can be estimated with standard thiosulfate solution. For large amounts, the weighing of free selenium liberated as in *b*, is undoubtedly subject to least objection, but gravimetric methods are not possible with the amounts found in a reasonable sample of most plants or soils. The method used for many analyses in the United States Department of Agriculture Bureau of Chemistry and Soils (Williams, 1937) depends upon the color developed when free selenium is liberated under such conditions that it forms a comparatively stable suspension. In the present work, fair success was had with this method, but the tendency of the suspended material to assume any color from pink through brown to black made comparison with a standard very uncertain. Addition of gum arabic (Association of Official Agricultural Chemists, 1935) made no decided improvement. Other workers have had the same kind of difficulties (Mathews, Curl, and Osborn, 1937).

Preliminary experiments indicated that the reaction, $H_2SeO_3 + 4K^+ + 4I^- + 4H^+ \rightarrow Se + 2\,I_2 + 3H_2O + 4K^+$, with consequent titration of the free iodine with standard thiosulfate, is suitable for the iodometric determination of small amounts of selenium. This method was criticized by Gooch and Reynolds (1895), who were unable to get complete reduction in the cold. However, Norton (1899) showed that a large excess of iodide in approximately 0.5 *N* hydrochloric acid gave excellent results except with large amounts of selenium. Recently, Berg and Teitelbaum (1928) raised two more objections: (1) the liberated iodine is absorbed by the colloidal

selenium and does not react quantitatively with thiosulfate, and (2) the end point is obscured by the color of the colloidal selenium. These complications are serious when selenium is present in amounts of 500γ (0.5 mg) or over; but with 200γ (0.2 mg) of selenium or less, no serious difficulties have been encountered in several hundred titrations, though the end point is decidedly different from that of an ordinary iodometric titration.

Tests of Analytical Methods with Pure Selenium Compounds.—Selenium dioxide was prepared by treating black selenium with concentrated nitric acid, warming gently to remove most of the excess acid, and then cautiously subliming onto the bottom of a cold porcelain evaporating dish. The first crop of crystals was treated with a drop of nitric acid and resublimed. The final product consisted of snow-white needles which were somewhat hygroscopic. A stock 0.1 *M* solution of selenious acid was prepared gravimetrically and diluted to 0.010 or 0.001 *M* as required. The reagents used were all of C.P. grade.

The purity of the contents of a stock bottle of sodium thiosulfate crystals was determined by numerous titrations against a standardized dichromate solution. The results were almost theoretical. Hence, for routine work, 0.05 *M* thiosulfate solutions were made at frequent intervals by weighing out the crystals, adding to freshly distilled water, and thereafter protecting from light and air. Each morning a fresh 0.001 *M* thiosulfate solution was prepared by dilution.

The usual precautions were taken to ensure that the potassium iodide did not liberate free iodine when put into solution. From a bottle of satisfactory crystals, a small volume of 25 per cent solution was made each morning. A small pinch of sodium bicarbonate was used to stabilize the solution, which also was protected from strong light.

The acid needed in the reaction proved to be the principal source of trouble. With high-grade hydrochloric or sulfuric acid at approximately 1 *N* concentration, no difficulty was encountered in securing zero blanks or stoichiometric reactions for a wide variation in the amount of selenium. However, the reaction had to proceed in the presence of nitric acid because that compound is the most convenient for oxidizing free selenium (formed as in step *b*, p. 127) to the tetravalent form. Two separate considerations are involved: (1) the strength of acid needed to enable the reaction between selenite and iodide to proceed at a satisfactory rate, and (2) the strength of nitric acid which can be tolerated by the excess iodide ion. These had to be studied together, for it was possible that the colloidal selenium produced in the first reaction had an effect upon the second one. Complete expulsion of the excess nitric acid by repeated

evaporation to dryness on a water bath, as suggested by Beath, Eppson, and Gilbert (1935), was tried; but in some cases loss of selenium occurred.

It is well known that the lower oxides of nitrogen react with iodide ion. Hence, care must be taken to ensure their absence. For micro-iodometric analysis, the color of nitric acid is not a safe guide. It has been found that vigorous aeration for 1 hour of any high-grade nitric acid except very brown lots suffices to remove the offending compounds. The distilled water used must be of high quality. A blank containing nitric acid, iodide, and starch should be tried each day in order to check the condition of the acid and of the water.

The most suitable amount of nitric acid for the iodometric determination of selenite was determined by adding 1.000 cc of 0.001 *M* selenious acid solution (79.2γSe) to the indicated volumes of concentrated nitric acid in a small Erlenmeyer flask and adjusting the volume to 10 cc; 0.25 cc of 25 per cent potassium iodide solution was added; and the flask was stoppered and allowed to stand for 5 minutes. The liberated iodine was then titrated with standard 0.001 *M* sodium thiosulfate solution. Starch solution was used in the ordinary way. After titration, the solutions were set aside and retitrated 10 minutes after introduction of the iodide. The titrations of the solutions containing the different volumes of nitric acid were as follows:

Nitric acid, in cc	Thiosulfate solution, in cc: 5 minutes	Thiosulfate solution, in cc: 10 minutes
0.05	0.600	0.740
0.10	0.680	0.950
0.20	2.447	2.780
0.40	3.700	3.850
0.60	4.020	4.078
0.80	4.090	4.175
1.00	4.191	4.222
1.40	4.232	4.312
1.60	4.380	4.445

The results show that 0.60 to 0.80 cc nitric acid give satisfactory results for the titration at 5 minutes and no serious error after 10 minutes. The theoretical volume of thiosulfate solution is 4.000 cc.

Since concentrated nitric acid is approximately 15 *N*, the desired concentration in the reaction vessel (0.60 to 0.80 cc in 10 cc) is about 0.9 to 1.2 *N*. Concentrations of nitric acid much higher than this slowly liberate free iodine from the excess iodide, as is shown by the data above.

The accuracy to be expected from the direct titration of varying

amounts of selenium was determined by titrating known amounts of selenious acid solution. The average results were:

Se added, γ	Se found, γ
4	5
7.9	8.4
15.8	16.9
31.6	33.8
47.5	49.8
63.3	66.1
79.2	82.0
158.4	161
237.6	241

With the proper conditions ascertained for the iodometric determination of soluble tetravalent selenium, the next step was to study the conditions necessary for the quantitative reduction of selenite, separation of the free element, and its reoxidation to the tetravalent state. Noyes and Bray (1927, p. 323) suggested the reduction of Se^{-4} by hydroxylamine-hydrochloride in approximately 5 *N* hydrobromic acid solution at steam-bath temperature. Preliminary experiments with selenious acid showed the same conditions to be satisfactory when hydrochloric acid was substituted for the hydrobromic. The characteristic brick-red precipitate is obtained except with very small amounts of selenium or when interfering substances are present. However, the change to the compact black form may occur very rapidly, so that the color is not a sure indication of the true conditions. The procedure adopted after many tests was: The solution, held in a 25 $\times$ 150 mm test tube, is made 4–6 *N* with hydrochloric acid in a total volume of 15–20 cc. It is placed in a boiling water bath. When it is hot, 2 cc hydroxylamine hydrochloride solution are added. Heating is continued for 1 hour with the addition of 1 cc more of the hydroxylamine solution. Centrifuging is done either at once or later. Addition of a very little finely divided asbestos aids in collecting the precipitate. If the asbestos is a thoroughly washed high-quality product, it does not affect the subsequent titration.

For the separation of the selenium from the liquid, two types of stick filter have been used with equal satisfaction. The first consisted of a capillary tube, the bore of which was slightly enlarged at the lower end. This swelling was filled with finely divided asbestos pulp. When connected with a suction, the asbestos became very tightly packed but still allowed liquid to pass with reasonable speed, 0.5 to 1.0 cc a minute. The other filter had a larger opening at one end which was filled with finely ground glass sintered in place. When a very thin pad of asbestos was pulled over this by suction, rapid filtering was possible without loss of

selenium. During the process of filtering, the inside of the test tube should be washed down several times with water, so that eventually all soluble materials are removed and the selenium is left with practically no water.

To oxidize the selenium, 0.8 cc of concentrated nitric acid is carefully run down the inside of the test tube and the outside of the stick filter. The tube is then placed in a steam bath for about 5 minutes and is turned about until the hot acid has had an opportunity to dissolve all the selenium present. The filter stick is put into a 125-cc Erlenmeyer flask and washed inside and out with water. It is then laid aside, and the contents of the test tube are washed quantitatively into the flask. With the total volume of liquid about 10 cc, the flask is placed on a sand bath and heated just to boiling. Spattering must be avoided. Vapors are removed by blowing out the flask two or three times with the breath. The flask is then cooled in running tap water, the contents adjusted to approximately 10 cc, and a stream of nitrogen or carbon dioxide is bubbled through for 5 minutes; 0.25 cc of 25 per cent potassium iodide solution is next added, and the stoppered flask is allowed to stand for 5 minutes. Titration is then made with 0.001 *M* sodium thiosulfate solution and 1 per cent starch solution.

The use of a very little asbestos to aid in collecting the selenium and in filtering out the liquid does not interfere with the titration; larger amounts obscure the end point. The color change involved in the titration is somewhat different from that of an ordinary iodometric determination, for the liberated selenium gives a golden color to the solution which is largely obscured by the iodine but becomes evident just at the end point. The reason for heating the solution to boiling, blowing out vapors, and flushing with an inert gas is chiefly to remove the reduction products of nitric acid, which react with iodide ion. Replacement of the air in and above the liquid is advisable also because the liberation of iodine by oxygen is thereby minimized. When the above process is carried out carefully, the amount of nitric acid finally left is approximately 0.6 cc, which, as shown earlier, is suitable for the reduction of selenite and titration of the liberated iodine.

The results of many experiments with different amounts of selenium carried through the processes of precipitation, reoxidation, and titration are summarized as follows:

Se added, γ	Se found, γ Range	Se found, γ Average
19.8	19.0–20.0	19.5
29.6	38.2–41.8	39.5
79.2	70.6–78.5	76.7
158.4	152 –157	154.6

Recovery was satisfactory over the range studied, which covered nearly all of the subsequent analyses on fruit and soils.

Analysis for Selenium in Plant Material.—Before the accuracy of recovery of selenium added to fruit could be studied, it was necessary to decide upon a method for destroying the organic matter contained in the skin and pulp of the fruits concerned without loss of selenium. The numerous methods which have been described for wet (Robinson *et al.*, 1934; Williams and Lakin, 1935) or dry (Mathews, Curl, and Osborn, 1937) combustion of plant material are time-consuming and are not without objection when selenium is to be determined quantitatively, for the free element and several of its compounds are readily volatilized. In an effort to prevent such loss, attention was directed to the simplified Karns (1932) apparatus devised by von Kolnitz and Remington (1933). In this, within a closed system, a cartridge of the dried vegetable material is thrust slowly into an oxygen flame and the products of combustion are absorbed in a train of wash bottles. This proved so successful that most of the materials reported on below were burned by that procedure. Two $1\frac{1}{2} \times 8$ inch washing tubes containing about 4 inches of distilled water above coarse Pyrex sintered glass filters fused in near the bottom were used to catch the products of combustion. Tests proved that most of the selenium was trapped in the first tube and that none escaped the second.

Charring and volatilization of carbohydrates before they reached the flame was a source of trouble with materials such as raisins. In seeking to obviate this condition, a method of dropping separate pellets of the material to be burned into a combustion dish was devised. It proved to be so successful that the first method was abandoned completely. The improved apparatus is shown in figure 1.

Fruit was divided as necessary, weighed, cut into thin slices, and dried in deep porcelain dishes on a water bath until most of the moisture was gone. The time required varied greatly, but 6 to 12 hours sufficed for most materials. Drying was then completed by placing the sample in a vacuum oven at 80°–90° C for a few hours. Nonhygroscopic material was then ground through a meat grinder with the fine disk attached and pellets weighing about 1 gram each were wrapped in onionskin paper. Ripe grapes absorb moisture from ordinary air with great rapidity and become sticky to handle. This may be avoided by grinding and wrapping in a coldroom in which the humidity is low.

The process of burning dried material is described best with reference to figure 1, which shows the main parts of the apparatus. A first pellet is placed in the combustion dish *A*, and a tail of the wrapping paper is ignited. Chamber *C*, with more pellets in the side arm, is placed in posi-

tion and the oxygen flow and suction are started. These must be so regulated that combustion is clean, no gas escapes through the water seal *K*, and frothing does not become excessive in the absorbers *L* and *L′*. A flowmeter in the oxygen inlet tube is very useful. Each pellet should be burned to a white ash before the next one is introduced. This is done by

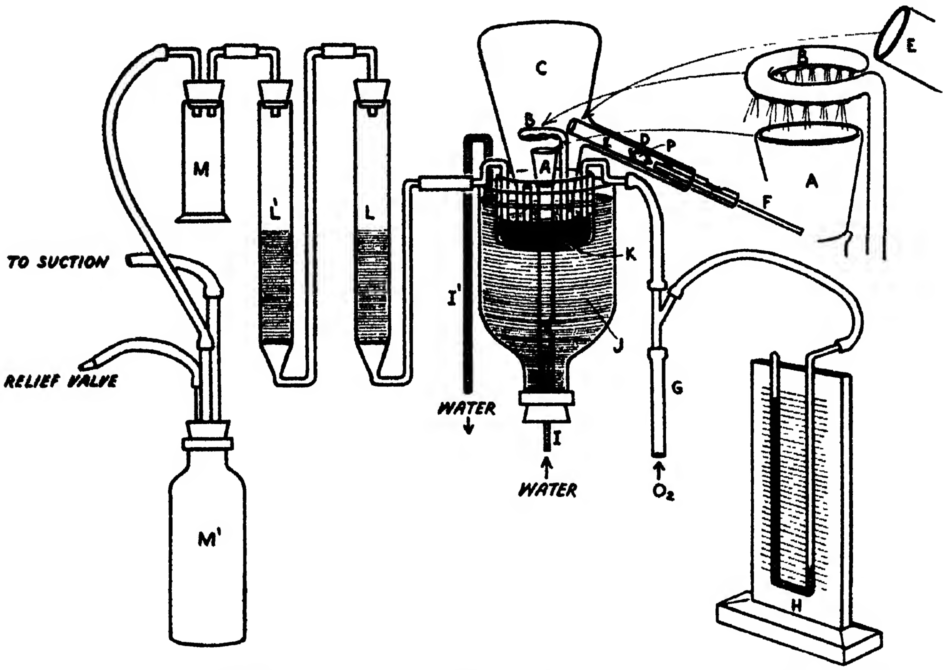

Fig. 1.—Apparatus for combustion of samples: *A*, combustion dish; *B*, ring burner made of Pyrex glass; *C*, combustion chamber; *D*, side arm; *E*, cartridge holder; *F*, rod; *G*, tube for introduction of oxygen; *H*, flowmeter; *I*, *I′*, tubes for circulating water through outer jacket, *J*; *K*, water seal; *L*, *L′*, absorbers; *M*, *M′*, traps; *P*, pellet in holder.

pushing in the rod *F* until the next pellet drops into the dish. If one loading of the side arm is not sufficient, more pellets may be placed in it by withdrawing rod *F*. The temperature of the dish remains high enough for some time to ignite the next pellet. Excessive heating of the water seal and of the inlet and outlet tubes is avoided by maintaining a continuous flow of tap water through the cooling jacket *J*, which is simply the upper part of a large acid bottle.

The success of a combustion is indicated by the color of the interior of the flask and of the water in the absorbers. If these remain relatively colorless, the organic matter is nearly all oxidized. On the other hand, much tarry or resinous matter will prevent the successful isolation of selenium.

When a burning is ended, the combustion chamber is allowed to cool while the contents of the absorbers are washed into a 250-cc distillation flask which connects by means of a ground-glass joint to a condenser. The inside of the combustion chamber *C* is rinsed with a small amount of hot nitric acid, then by distilled water, the combustion dish is dropped into the distillation flask, any ash which adheres to the inlet or outlet tubes is scraped into the water seal, and the tubes are washed with a very small quantity of hot nitric acid to dissolve any selenium on them. Lastly, the

TABLE 3

Recovery of Selenium Added to Plant Material

Selenium added		Plant material	Selenium found	Per cent recovery
Amount	Form			
γ			γ	*per cent*
25	Selocide	Grapes	23	92
25	Selocide	Grapes	22	88
32	Selenite	Citrus pulp	26	81
55	Selocide	Citrus rind	58	105
60	Selenite	Citrus pulp	57	95
62	Selenite	Grapes	45	73
64	Selocide	Citrus rind	57	89
65	Selenite	Grapes	52	80
66	Selocide	Citrus pulp	57	86
72	Selenite	Citrus rind	61	85
76	Selenite	Citrus rind	78	103
79	Selenite	Grapes	69	87
79	Selenite	Barley straw	77	97
82	Selenite	Grapes	71	87
82	Selenite	Citrus pulp	85	104
158	Selenite	Citrus pulp	146	92

water seal with everything which has fallen into it is transferred to the distillation flask. A little hydrobromic acid is added first to destroy the excess nitric acid. Then bromine and hydrobromic acid are added, and the selenium is distilled as in the method of Robinson *et al.* (1934). The distillate is treated with sulfur dioxide and hydroxylamine hydrochloride to precipitate the selenium. The rest of the process is as described for the precipitation, reoxidation, and titration of pure selenite solutions.

The results obtained in sixteen runs with varying amounts of selenium added to different plant materials are shown in table 3. The recovery with grapes (average 84.5 per cent) was distinctly lower than with citrus (average 94.5 per cent). This is due at least in part to the fact that this portion of the work with grapes was done before the improved method of combustion was used, and in several cases combustion was very poor. While, on the basis of these results, correction factors might be applied

to the analyses of material from the field, they would have no effect whatsoever upon the significance of the results and hence are not used. Data in the subsequent tables often is given to two decimal places as calculated from the titration figures. This undoubtedly exceeds the accuracy of the results, but it is felt that they are known to the first decimal.

Through the courtesy of H. G. Byers, a sample of narrow-leaf milk vetch, *Astragulus pectinatus,* from Kansas, was secured. The average selenium content from several closely agreeing analyses was found to be 1,407 p.p.m. Byers (1936) has reported this sample to contain 1,750 p.p.m. Se. The finely ground sample had a very strong repulsive odor, which is characteristic of plant materials that lose a volatile selenium compound when dried (Beath, Eppson, and Gilbert, 1937); and since about a year elapsed between analyses in the two laboratories, a decrease in selenium content would be expected. The good agreement of the repeated analyses in the present work is an indication that the method is applicable to highly seleniferous plant material.

Analysis for Selenium in Soil.—For the separation of selenium from soils, the bromine-hydrogen bromide distillation method of Robinson *et al.* (1934) was used. Since this is a recognized method, only a few tests with known amounts of added selenium were made to ensure that the technique was correct. The distillate which contained selenium as selenious bromide was put into a boiling water bath, saturated with sulfur dioxide, and treated with hydroxylamine hydrochloride. The separation of the free selenium and its reoxidation and titration were carried out as described earlier.

SELENIUM IN AND UPON GRAPES AND CITRUS

Grapes.—Numerous sprays containing Selocide were applied to grape vines during the months of May, June, and July. Fruit was harvested in September and analyzed for selenium content. The results indicated that considerable variation occurred in the distribution of selenium. The following data cover the more important spray mixtures used. All results in this section are expressed in terms of fresh weight.

Treatment	Date of spraying	Se, p.p.m. (fresh weight)
Selocide 1:400 + 4 oz. blood-albumin spreader to 100 gals.	June 9	0.5
	July 7	2.5
Selocide 1:600 + 4 oz. blood-albumin spreader to 100 gals.	June 23	0.3
	July 7	1.8
Selocide 1:400 + 8 oz. Fluxit to 100 gals.	July 7	0.7
Selocide 1:600 + 8 oz. Fluxit to 100 gals.	July 7	0.7
Selocide 1:600 + liquid soap 1:400	June 23	0.8
Selocide 1:800 + tank-mix oil 1:400	May 22	1.0
Selocide 1:800 + 4 oz. Fluxit, 4 oz. blood-albumin spreader, and 5 lbs. sulfur to 100 gals.	July 12	0.5
Selocide 1:100 + 4 oz. Fluxit, 4 oz. blood-albumin spreader, and 5 lbs. sulfur to 100 gals.	July 12	0.4
None	.....	0.11
	.....	0.08
	.....	0.20
	.....	0.06
	.....	Av. 0.11

The different wetting powers of the sprays and the varying stability of Selocide in them make impossible any close correlation between concentration of selenium in the sprays and the amounts left upon the grapes. However, it appears that a considerable deposit resulted from the more concentrated sprays and that early applications left but little selenium, probably because both fruit growth and loss by weathering are marked in the early part of the season.

A few preliminary experiments were carried out to test the absorption by grapes of selenium applied to the soil. In each of these tests, on March 15, 2 gallons of solution containing the specified amount of a selenium preparation were poured about a vine over a circle of about 3-foot radius. All fruit was picked for analysis on July 24. The results showed that the grapes from vines treated with 1 pint Selocide (23.6 grams Se) or with 36.7 grams of sodium selenite (17.6 grams Se) contained 0.05 p.p.m. Se, which was the same as that in grapes from an untreated vine. The application of 5 pints of Selocide (118.2 grams Se) resulted in the occurrence

of 0.16 p.p.m. Se in the fruit. Evidently no marked absorption occurred during the interval March 15 to July 24.

Citrus.—Selocide sprays have been applied at intervals to certain areas of orange and lemon groves since 1932. During the spring and summer of 1935, samples of fruit from several of these groves were analyzed for selenium content. The results are given in table 4.

In an attempt to determine if selenium is absorbed from the soil after large amounts of Selocide have been applied to the ground about lemon trees, 520 cc of Selocide in 22 gallons of water were applied to a depth of 18 inches in a 12-foot square on September 3, 1933. This amounted to 3 p.p.m. Se in the first 18 inches of soil. Fruit picked on March 10, 1935, contained 0.02 p.p.m. Se in the rind and none in the pulp. A similar treatment, except that 1,732 cc Selocide were used in 34 gallons of water (10 p.p.m. Se in the soil) resulted in the following amounts of selenium in the fruit picked March 10, 1935: rind, 0.31 p.p.m.; pulp, 0.05 p.p.m. Fruit from an untreated tree in the same grove had 0.07 p.p.m. Se in the rind and none in the pulp, and that from another untreated tree: rind, 0.06 p.p.m.; pulp, 0.03 p.p.m. Se.

Effects of Several Years' Spraying upon Selenium Content of Soil and Fruit.—The data given in the two preceding sections indicated that but little selenium occurs in citrus fruit or grapes as the result of ordinary spraying with Selocide over short periods. Since the material had been applied to certain groves and vineyards for several years, both soil and fruit from these localities were analyzed to obtain information on the distribution of selenium in the soil and its absorption into fruit.

During the summer of 1937, fruit and soil samples were taken from eight citrus groves which had been sprayed to varying extents with Selocide. Similar samples were taken from other parts of the same or neighboring groves which never had been treated with selenium. In each case, the corresponding samples were from localities of the same soil type and treated by the same irrigation and other cultural practices. The exterior of each fruit used for analysis was washed in nitric acid to remove any adhering selenium. A preliminary account of the analytical results has already been published (Hoskins, 1938). The complete data are given in table 5.

The soils were classified by M. R. Huberty, Associate Irrigation Engineer in the Experiment Station. Additional information concerning their origin has been furnished by C. F. Shaw, Soil Technologist in the Experiment Station, Berkeley, as follows:

The Altamont soils were formed by the weathering in place of marine sandstones

TABLE 4

SELENIUM OCCURRING IN AND UPON CITRUS AS A RESULT OF SPRAYING WITH SELOCIDE

Lot No.	Treatment: Material	Treatment: Date	Remarks: Maturity and variety of fruit and time from last spraying to analysis	Selenium (on basis of fresh weight): Rind	Pulp	Whole fruit
			Oranges			
				p.p.m.	*p.p.m.*	*p.p.m.*
1	Selocide Selocide 1:800+⅓ per cent oil	Summer 1933 Summer 1934	Mature Valencias, 9 months	0.27	0.06	0.11
3a	Selocide 1:800+⅓ per cent oil	June 18, 1934	Mature Valencias; 9 months	0.06	0.03	0.04
3b	Selocide 1:800+⅓ per cent oil	June 18, 1934	Half-grown Valencias*	0.00	0.00	0.00
3c	Selocide 1:800+⅓ per cent oil	June 18, 1934	Mature Washington Navels; 9 months	0.05	0.03	0.04
5a	Selocide 1:800+⅓ per cent oil and 3 lbs. cryolite	July 4, 1934	Mature Valencias; 9 months	0.32	0.07	0.12
5b	Selocide 1:800+⅓ per cent oil and 3 lbs. cryolite	July 4, 1934	Green Valencias*	0.00	0.02	0.01
6a	Selocide 1:800+⅓ per cent lime-sulfur Selocide 1:800+⅓ per cent oil and 3 lbs. cryolite	Aug. 22, 1934 June 1, 1934	Mature Valencias, 9 months	0.18	0.00	0.05
6b	Selocide 1:800+⅓ per cent lime-sulfur Selocide 1:800+⅓ per cent oil and 3 lbs. cryolite	Aug. 22, 1933 June 1, 1934	Green Valencias*	0.01	0.05	0.04
10	Selocide 1:800+⅓ per cent oil	Dec 9, 1933	Mature Valencias*	0.00	0.02	0.02
13c	Selocide 1:800+⅓ per cent oil Selocide 1:800+⅓ per cent oil and ⅓ per cent lime-sulfur	July 7, 1934 Mar 30, 1935	Mature Valencias; 6 weeks	0.8	0.03	0.25
13d	Selocide 1:800+⅓ per cent oil Selocide 1:800+⅓ per cent oil and ⅓ per cent lime-sulfur	July 7, 1934 March 30, 1935	Put through regular packing-house treatment (no waxing), 36 per cent of Se removed from rind	0.5		
14a	Selocide 1:800+⅓ per cent oil	May 7, 1935	Mature Valencias; 1 week	0.41	0.01	0.11
14b	Selocide 1:800+⅓ per cent oil	May 7, 1935	Put through regular packing-house treatment (no waxing); 68 per cent of Se removed from rind	0.13		..
15a	Selocide 1:800+⅓ per cent oil	July, 1934	Mature Navels; 10 months	0.05	0.03	0.03
15b	Selocide 1:800+⅓ per cent oil	July 1934	Put through regular packing-house treatment (no waxing), 20 per cent of Se removed from rind	0.04		

			Grapefruit			
				p.p.m.	*p.p.m.*	*p.p.m.*
5	Selocide 1:600+1/3 per cent oil	Dec. 9, 1934	4 months, red Se visible on lower surface	0 83	0 02	0.46
			Lemons			
				p.p.m.	*p.p.m.*	*p.p.m.*
2	Selocide 1 800+1/3 per cent lime-sulfur	Nov 7, 1934	Mature; 4 months	0 72	0 02	0.26
2*a*	Selocide 1 800+1/3 per cent lime-sulfur	Nov 7, 1934	Mature, 4 months	0 80	0 01	0.27
5	None		Half-grown	0 06	0 03	0.04
9	Selocide 1.600 Selocide 1 600 Selocide 1 600 Selocide 1·800 Selocide 1 800	Oct 31 1932 Mar. 21, 1933 July 8, 1934 Mar. 30, 1935 Oct 10 1935	Mature, 3 months	1 95	0 21	0.63
2	Selocide 1.800+1/3 per cent oil	July 7, 1934	Mature, 8 months	0 16	0 02	0.06
7	Selocide 1 800+1/3 per cent oil	April 11 1934	Mature: 11 months	0 06	0 01	0.03
8	Selocide 1 600+1/3 per cent oil	Dec. 9, 1933	Mature*	0 00	0 06	0 03
13*a*	Selocide 1 800+1/3 per cent oil Selocide 1 800+1/3 per cent oil and 1/3 per cent lime-sulfur	July 7, 1934 Mar 30 1935	Mature, 2 months	1.30	0 10	0.52
13*b*	Selocide 1 800+1/3 per cent oil Selocide 1 800+1/3 per cent oil and 1/3 per cent lime-sulfur	July 7, 1934 Mar 30, 1935	Put through regular packing-house process; 29 per cent of Se removed from rind	0 90		

* Fruit not on tree when spray was applied to tree.

TABLE 5

RESULTS OF ANALYSIS OF FRUIT AND SOIL FROM CITRUS GROVES, 1937

Plot	Fruit	Soil type	Applications of Selocide	Se in soil				Se in fruit		
				0–6 in.	6–12 in.	12–24 in.	24–36 in.	Skin*	Pulp*	Whole fruit (av.)
				p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
A	Lemons	Yolo clay loam	October, 1932, 1:600 March, 1933, 1:600 July, 1934, 1:600 March, 1935, 1:800 October, 1935, 1:800 January, 1936, 1:800	2.08	1.29	0.76	0.29	0.09 0.08	0.07 0.08	0.08
AA	Valencia oranges	Yolo clay loam	None	0.43	0.22	0.28	0.23	0.17 0.09	0.09 0.17 0.10	0.12
B	Valencia oranges	Hanford fine sandy loam	Five during 1933 to 1935 at 1:600 and 1:800, last in Apr. or May, 1936	1.28	0.42	0.26	0.27	0.53 0.42	0.11 0.03	0.17
BB	Valencia oranges	Hanford fine sandy loam	None	0.51	0.24	0.27	0.22	0.04 0.09	0.04 0.03	0.04
C	Lemons	Hanford stony sandy loam	October, 1934, 1:800 March, 1935, 1:800 January, 1936, 1:800 February, 1936, 1:800 November, 1936, 1:800	2.16	0.67	0.60	0.39	0.31 ...	0.07 0.05	0.12
CC	Lemons	Hanford stony sandy loam	None	0.39	0.23	0.25	0.25	0.14 0.11	0.04 0.14	0.10
D	Valencia oranges	Hanford gravelly sandy loam	January, 1935, 1:800 January, 1936, 1:800 May, 1936, 1:800	1.50	0.68	0.41	0.34	0.26 0.18	0.12 0.12	0.16

DD	Valencia oranges	Hanford gravelly sandy loam	None	0.41	0.25	0.28	0.29	0.09 0.12	0.03 0.02	0.05
E	Valencia oranges	Altamont clay	July, 1933, 1:600 June, 1934, 1:600 April, 1936, 1:600	0.48	0.26	0 24	0.20	0.37 0.08 0.06	0.05 0.10	0.10
EE	Valencia oranges	Altamont clay	None	Composite, 0.35				0.00 0.18	0.01 0.07	0.05
F	Lemons	Yolo silt loam	September, 1933, 1·600 August, 1934, 1:800 March, 1935, 1.800 October, 1935, 1 800 November, 1935 1 800	1 07	1.10	0 86	0.86	0.12 0.25	0.02 0.03	0.07
FF	Lemons	Yolo silt loam	None	Composite, 0 60				0.13 0 03 0 15	0.01 0 02	0.05
G	Lemons	Yolo fine sandy loam	Spring of 1936, 1:800	0 37	0 15	0 18	0 21	0 34 0 12 0.15 0 29	0.03 0.04	0.10
GG	Lemons	Yolo fine sandy loam	None	Composite, 0 12				0 04 0 12	0.01 0.01	0.04
H	Valencia oranges	Yolo loam	June, 1934, 1 1,000 August, 1934, 1·800	0 85	0 72	0 54	0.31	0 08 0 10	0 01 0 04	0.05
HH	Valencia oranges	Yolo loam	None	Composite, 0 31				0 03 0 09	0 02 0.02	0 04

* Data are for duplicate or replicate tests on fruit from the various plots

and shale rocks, in this case of marine origin, which have not been leached to any great extent.

The Hanford soils were derived from stream deposits on alluvial fans and have their origin from granitic rocks.

The Yolo soils were formed from stream deposits on alluvial fans and flood plains and are derived from a variety of sandstones and shales which are mainly of marine origin.

The results with Thompson Seedless grapes are given in table 6.

TABLE 6

RESULTS OF ANALYSIS OF FRUIT AND SOIL* FROM VINEYARDS, 1937

Plot	Application of Selocide	Se in soil				Se in grapes (ripe)	
		0–6 inches	6–12 inches	12–24 inches	24–36 inches	Un-washed	Washed
		p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
1	None	0.32	0.25	0.24	0.23	0.11 0.06	
2	1933, 1:400 1934, 1:400 1935, 1:600 1936, 1:600 1937, 1:600	1.00	0.49	0.41	0.33	1.80† 0.80	0.67
3	1933, 1:400 1934, 1:400 1935, 1:600 1936, 1:600 1937, 1:600	1.51	0.63	0.61	0.30	0.61 0.65	0.26
4	1933, 1:400 1934, 1:400				.	0.14	
5	1935, 1:600 1936, 1:400	.				0.23	

* These soils were all clay loams, but the particular types have not been determined as yet.

† The wide variation between these two figures is an indication of unevenness in application of the spray. Free selenium could be seen upon the grapes which had the larger residue.

THE NATURAL OCCURRENCE OF SELENIUM

The results reported for citrus and grapes in the foregoing section indicate that the amounts occurring in or upon these products as the result of the proper use of Selocide are a few hundredths of a part per million in most cases. While this may be taken as an indication that a hazard either to the plants or to animals or humans is not likely to result from the use of this spray material, an examination of the available information on the natural exposure of plant and animal life to selenium and

its effects thereon is advisable before conclusions are drawn. In the following sections, published data are collected and certain experimental results of the present investigation are mentioned.

Abundance of Selenium.—Although selenium is ordinarily considered to be a comparatively rare element, it ranks about fiftieth in abundance among the elements making up the crust of the earth (Noddack and Noddack, 1930, 1934) and is more abundant than iodine and nearly as common as arsenic. Its close association with sulfur is indicated by the fact that it was first found in the flue dust of a sulfuric acid plant. Chemically, selenium and sulfur are much alike, and they form analogous series of compounds. According to Goldschmidt and Hefter (1933), sulfur is oxidized somewhat more readily than selenium, so that in the course of geologic time, a larger fraction of the selenium tends to remain in place as the free element, whereas sulfur is oxidized to soluble form and removed. This condition, together with the strong adsorption of selenium by hydrated iron oxide (Goldschmidt and Strock, 1935), largely explains the changes from the original selenium : sulfur ratio which have come about in various parts of the earth's crust.

Selenium in Soils.—Selenium has been found to occur in practically all soils which have been examined for its presence. The concentration varies widely but may be correlated in a general way with the geological origin of the various soils (Byers, 1935; Strock, 1935). Thus, that from sedimentary deposits contains most of the selenium which has been carried into the sea by rivers. Ocean water is low in selenium content—for example, 0.004 p.p.m. near Helgoland—and the Se:S ratio in salt deposits from ancient seas is low (Goldschmidt and Hefter, 1933).

An examination of Hawaiian soils led Byers, Williams, and Lakin (1936) to the conclusion that the selenium therein was of volcanic origin. They suggested that the original source of selenium now occurring in many sedimentary formations in the central United States was volcanic gases and dust from the numerous volcanoes of the Upper Cretaceous period. After entering the oceans, which at that time covered most of the present area of this country, the selenium was soon combined with iron compounds and carried to the bottom. The various sedimentary layers differ in their content of selenium according to the volcanic activity at the time of their deposition. That known as the Pierre shale, which extends over parts of nearly a dozen states in the Midwest and Rocky Mountain region, is exceptionally rich in this element.

In soils from magmatic rocks, the selenium is due mainly to that which was originally associated with the metallic sulfides. Beath, Gilbert, and Eppson (1937) have suggested that intrusions of such materials may

account for the selenium now present in the sedimentary rocks and their derived soils in Wyoming.

A further factor influencing the distribution of selenium is the dissolving of its compounds in underground waters and passage as solutes to locations favorable for reprecipitation.

The net result of all these processes is that the distribution of selenium is world-wide but very far from uniform. The free element is very insoluble, but at least the red allotropic form is converted in the presence of water and mild oxidizing agents into soluble combined states (Montignie, 1934; Calcagni, 1935); and also, certain bacteria are able to effect the oxidation of the free element (Lipman and Waksman, 1923).

The compounds of selenium are, on the whole, at least as soluble as those of sulfur, and leaching by either naturally or artificially applied water may be expected to reduce the selenium content of a soil provided the drainage is such that percolation of water through the soil occurs readily. This effect will not necessarily be shown immediately but regions of habitually plentiful rainfall or long-continued heavy irrigation must eventually come to the condition of minimum soluble selenium and usually low total selenium in the soil. This is particularly true of sandy soils, less so with heavy clays through which water moves with difficulty. The Belle Fourche Irrigation Project in South Dakota has been cited by Byers (1935) as an example of the effect of irrigation for twenty-five years. In this area, the selenium content of the soils is only a little less than that in neighboring unirrigated districts, but that of the vegetation was uniformly low.

The forms in which selenium occurs in the soil have not been investigated with thoroughness, but they may be divided in general into organic and inorganic. On account of the completeness with which hydrated iron oxide removes selenium from solution, one may reasonably suppose that some of it is present as a very insoluble iron selenide or basic selenite (Williams and Byers, 1936). The more soluble inorganic portion may be present as calcium selenite or selenate. Electrodialysis experiments (Franke and Painter, 1937) have shown that selenium added to soil as sodium selenite in large part became nondialyzable. Previous destruction of the organic matter in the soil by heating to 500° C did not prevent binding of added selenium. Selenate is much less easily fixed by the soil. The probable nature of the organic compounds of selenium and their special significance with regard to plant absorption will be considered later.

Only very fragmentary data are available regarding the selenium content of soils in various parts of the world, and, in fact, only in the United

States has a start been made toward a survey of the situation. The results summarized by Byers (1935, 1936) show that selenium occurs in South Dakota, Nebraska, Kansas, Oklahoma, Montana, Wyoming, Colorado, Utah, New Mexico, Arizona, and the Hawaiian Islands. In South Dakota every sample taken in an area of about 500,000 acres contained selenium in amounts varying from a trace to 40 p.p.m. The highest selenium content so far reported for a soil in the United States (Logan County, Kansas) is 82 p.p.m., but only 10 feet away it was but 16 p.p.m. Such varia-

TABLE 7

SELENIUM CONTENT OF SOILS AT VARIOUS DEPTHS

Depth	Niobrara clay lime*		Selenium in "poison area" in Colorado†
	Selenium in Horizon A	Selenium in Horizon B	
inches	*p.p.m*	*p.p.m.*	*p.p m.*
0–12	6.0	1 0	1 4
12–24	1 0	0 7	1 1
24–36	0.8	0.5	2 6
36–48	1.0	0 5	6.4
48–60	0.8	0 5	17 8
60–72	0.8	0.7	4 7

* From Byers (1936), p. 39.
† From Beath, Eppson, and Gilbert (1937).

tions occur not only from point to point but in profiles. Thus Niobrara clay lime in Kansas and a known "poison area" in Colorado showed considerable variation of selenium with depth (table 7).

A few data for Hawaiian soils are particularly interesting because they range in selenium content from 0.4 p.p.m. to 26.0 p.p.m. with no decrease in the regions in which there are as much as 120 inches of rain yearly (Byers, Williams, and Lakin, 1936). The explanation offered for this absence of leaching is that the soils contain much iron, and hence the selenium probably is held in an insoluble complex combination. The idea that selenium will be low in regions having 20 inches or more of rain (Wilcox, 1935) is thus shown not to be applicable everywhere, but it is true that abundant rainfall leads to low amounts of available selenium.

Selenium in Water.—An indication of the effect of increased drainage in removing soluble selenium from the soil is given by the analysis of certain drainage waters of the Belle Fourche Irrigation Project (Byers, 1935). The water from a tile drain installed in 1916 and in use ever since contained 0.08 p.p.m. Se, whereas that in a drain installed in 1933 for the purpose of improving the movement of water from a certain area contained 1.2 p.p.m. Further illustration of the effect of irrigation is af-

forded by analyses of water in drainage ditches which empty into the Colorado River in Colorado. The selenium content varied from 0.32 to 2.68 p.p.m. (Williams and Byers, 1935). Above the discharge point of these ditches, the water of the Colorado River was free of selenium. Several tributaries were contaminated in a similar way. The result was the presence of 0.03 p.p.m. Se in the Colorado River near Grand Junction. Since irrigation has been practiced on a large scale in these districts for a comparatively short time, it is to be expected that large amounts of soluble selenium will be removed until that remaining is greatly reduced.

The water of shallow wells in the seleniferous areas of Nebraska and South Dakota has been found to contain as much as 0.2 p.p.m. Se, but deep well water is practically free of it. Similarly 0.56 p.p.m. Se was found in the water of a shallow well near Fallon, Nevada, but a deep artesian well nearby contained none (Byers, 1936). Of 44 samples of drinking water from wells in South Dakota and Nebraska examined by Smith and Westfall (1937) 23 per cent contained selenium in amounts from 0.05 to 0.33 p.p.m. No definite relation between depth and selenium content could be found. A so-called "poison spring" in South Dakota was found to have 0.4 p.p.m. Se in its water (Miller and Byers, 1935). It is interesting to note that selenium at a concentration of 0.2 p.p.m. was found in the medicinal waters of La Roche-Posay in the department of Vienne, France (Taboury, 1909).

Selenium in Plants.—Probably so reactive an element as selenium should be expected to be absorbed to varying extents by different kinds of plants. The facts so far determined reveal a truly amazing selectivity. On the basis of their tendency to accumulate selenium, plants may be classified into three groups, corresponding to (*a*) high, (*b*) moderate, and (*c*) slight absorption (Beath, Eppson, and Gilbert, 1935; Miller and Byers, 1937).

In group *a* are included certain species of *Astragalus* (family Leguminosae), especially *A. racemosus* and *A. bisulcatus* (poison vetch); *Stanleya pinnata* and *S. bipinnata* (family Cruciferae), and *Xylorhiza Parryi* (family Compositae). These plants grow more vigorously on seleniferous soil and may accumulate the element to the extent of a 1,000 p.p.m. or more: for example, Byers (1935, 1936) gives examples of *Astragalus bisulcatus* containing 2,000 to 4,000 p.p.m. Se growing on soils which contained from 0.8 to 3.0 p.p.m. Se. The highest selenium content so far reported (14,920 p.p.m.) is for a sample of *A. racemosus* growing on a Pierre shale which had 22.7 p.p.m. Se. No sign of injury was evident. Possibly the element plays a rôle in the physiology of these plants (Trelease and Trelease, 1938).

An extremely important result of the growth of such plants in a seleniferous region is the conversion of selenium from a relatively insoluble condition to one in which it is readily absorbed by many plans other than those of group *a*. Hence the latter have been called "selenium converters," or "indicators."

Not all species in a genus behave alike in selenium absorption. This contrast in the behavior of closely related plants is well illustrated by *Astragalus*. Thus, in five plots having a mean soil content of 2.1 p.p.m. Se, *A. bisulcatus* averaged 1,250 p.p.m. Se and *A. missouriensis* 3.1 p.p.m. Se. Further data are given by Byers and Knight (1935). Similar differences have been found in other families and genera.

In group *b* are included *Aster Fendleri* and *A. multiflorus* (family Compositae), several species of *Atriplex* (family Chenopodiaceae), and the ordinary cereals (family Gramineae). These plants are not able to concentrate large amounts of selenium from ordinary seleniferous soil, but if they grow near the spot at which a selenium-converting plant has grown, they may accumulate the element very markedly.

In an experiment reported by Beath, Eppson, and Gilbert (1937), wheat was grown on Steele shale soil through which green *Astragalus bisulcatus* was dispersed in the upper foot in such quantity that the average concentration of selenium was 10 p.p.m. At the conclusion of the growing season, the wheat contained selenium as follows: heads, 95 p.p.m.; stem and leaves, 123 p.p.m.; roots, 107 p.p.m. Another sample of the same wheat grown upon the same shale without the addition of the *Astragalus bisulcatus* contained only a trace of selenium. In the same experiment, wheat was raised on the same soil to which potassium selenite had been added at the rate of 10 p.p.m. selenium. In the matured wheat, selenium was present as follows: heads, 19 p.p.m.; stem and leaves, 17 p.p.m.; roots, 36 p.p.m. Thus it was shown that selenium from decomposed "converter plants" is much more readily taken up by other plants than is selenium in inorganic combination, although the difference may be due to failure of the selenium from plants to be rendered insoluble by reaction with metallic salts of the soil, for example, those of iron. Further consideration of the availability of selenium will be given in a later section.

Plants of group *b* are usually affected adversely by large amounts of selenium in their tissues. Thus Hurd-Karrer's (1935) data show that wheat grown on soil treated with sodium selenite showed injury when its content of selenium was from 300 to something over 600 p.p.m., calculated on an air-dry basis. Analysis of wheat from various parts of the world, including Argentina, Australia, Canada, Hungary, Mexico, New

Zealand, South Africa, and Spain (Robinson, 1936) showed selenium to be present in every sample in amounts from 0.1 to 1.9 p.p.m. The samples were obtained from the respective marketing centers and hence represent average conditions for large areas. Undoubtedly, wheat from restricted localities in other countries contains high amounts of selenium just as in the case of certain areas in the United States. Unfortunately, in the manufacture of flour, the toxic portion of grain is not lost: Horn, Nelson, and Jones (1936) found the toxic principle to be uniformly distributed in the milled fractions. Smith and Westfall (1937) have reported analyses of cereal grains from Nebraska and South Dakota which may be summarized as follows:

Grain	Selenium, p.p.m. Minimum	Maximum
Wheat	1.15	18.8
Corn	1.00	14.9
Barley	1.65	5.75
Oats	2.00	10.00
Rye	0.87	3.80

Hurd-Karrer (1935) has reported that mustard absorbs large quantities of selenium from soil containing 5 p.p.m. Se as sodium selenate. Accordingly, it was of great interest to determine if the common mustard, *Brassica campestris*, which comprises a large portion of the covercrop in the citrus district of California, absorbs much selenium from the soil of treated groves. Samples were selected from beneath and around the trees and found to contain from 1 to 4.7 p.p.m. Se, with no consistent difference between treated and untreated plots. This is a further indication that the residue of selenium from Selocide is not readily absorbed by plants. Of course, the possibility remains that some other plant species grown now or in the future in the citrus or vineyard districts of California, will prove to be a "selenium converter." A plant survey should be made for these districts similar to those already made in South Dakota, Nebraska, Wyoming, etc.

The plants of group *c*, which have a limited tolerance for selenium and are able to absorb but little of it even from highly seleniferous soils, include the gamma grasses, *Bouteloua gracilis* and *B. curtipendula*, buffalo grass, *Buchloe dactyloides*, and numerous other native range plants (Beath, Eppson, and Gilbert, 1935). Unfortunately, the data available as yet are largely limited to plants of the Rocky Mountain and Great Plains regions.

The question of absorption of selenium by alfalfa has received some attention. Since alfalfa is a member of the same family as *Astragalus*, it might conceivably be of the "selenium converter" type. From a clay loam

soil to which 4 p.p.m. Se had been added as sodium selenate, Hurd-Karrer (1937) found alfalfa to collect 220 p.p.m. Se (air-dry basis). Byers (1936) found 200 p.p.m. Se in a sample from an irrigated field whose soil contained but 0.7 p.p.m. Other samples on various other soils had up to 40 p.p.m. Several samples of alfalfa from soils of 0.5 p.p.m. Se contained none. Beath, Eppson, and Gilbert (1935) found only a few parts per million of selenium in alfalfa growing on seleniferous shales but were able to give more to samples of the plant by irrigating them with aqueous extracts of *Astragalus bisulcatus*. No sample of alfalfa from South Dakota analyzed by Moxon (1937) contained more than 10 p.p.m. Se. In the present investigation, dried alfalfa from the San Joaquin Valley was found to contain only a trace of selenium, whereas in the soil on which it was grown there were 0.4 p.p.m. The evidence indicates that alfalfa does not absorb really large amounts of selenium from soils containing it to the extent of several parts per million. Probably it belongs to group *b*, whose members take up large amounts of selenium only when the element has been rendered available by previous incorporation in a "selenium-converter plant." In the present investigation, melilotus clover was grown by D. R. Hoagland of the Plant Nutrition Division on soil to which Selocide had been added at the rate of 60 kg Se per acre. The dry plants were analyzed by P. L. Kirk of the Biochemistry Division and found to contain 0.3 p.p.m. Se. Apparently this clover does not absorb selenium readily.

Comparatively little data are at hand regarding absorption of selenium from the soil by truck crops. Plants grown on Keyport clay loam to which sodium selenate had been added to give 5 p.p.m. Se contained the following amounts of selenium in their leaves (Hurd-Karrer, 1937):

Plant	Selenium, p p m
Cabbage, young	344
Cabbage, old	180
Broccoli	220
Turnip	240
Pea	60
Corn	60
Spinach	50
Lettuce	50

Byers (1935) found the following amounts:

Plant	Selenium, p p m
Turnips	25
String Beans	2
Lettuce	7
Cabbage	100

Unfortunately, no information concerning the soil was given. Several kinds of vegetables, grasses, and cereals were grown for four seasons on soils from Steele and Niobrara formations containing 2–4 p.p.m. Se (Knight and Beath, 1937). The highest selenium content of the plants during the fourth season was 3.5 p.p.m. Apparently none of them are "selenium converters." The selenium contents of the edible portions of various vegetables, presumably grown in South Dakota or Nebraska, were reported by Smith and Westfall (1937):

Material	Selenium, p.p.m. Minimum	Selenium, p.p.m. Maximum
Cucumber	0.12	0.55
Potato	0.24	0.94
Beet	0.32	1.18
Tomato	0.18	1.22
Carrot	0.43	1.30
Pea, bean	0.38	2.04
Cabbage	0.23	4.52
Rutabaga	1.72	6.00
Onion	0.36	17.80

Since these data apparently refer to fresh weight, obviously some of the ordinary vegetables from seleniferous regions may contain as much selenium as the plants which have been classified into group *b*.

Additional data concerning the absorption of selenium by various vegetables was afforded by certain experiments conducted by Hoagland. The results of the analyses, made by Kirk, are summarized in table 8.

Extremely little published information is at hand regarding selenium in the fruit of trees. Byers (1936) found less than 0.1 p.p.m. Se in the pulp and skin of each of eighteen samples of apples from Colorado. The maximum selenium content of the apple seeds was 0.5 p.p.m., and many had only a trace.

The stage of growth may have an important effect upon the content of selenium in various plants. This matter has been investigated by Beath, Eppson, and Gilbert (1937) for several highly seleniferous plants. The following results are typical for those obtained with *Aster commutatis:*

Stage of growth	Month	Selenium, p.p.m.
Initial growth	May	590
Prebloom	June	273
Full bloom	August	233
Seeding	November	15

A decrease in selenium content with age cannot be taken as characteristic of all such plants, however, for *Oonopsis condensata* contained the maximum amount at the period of full bloom. Very little information on this

point is available for plants which are important foods for man or animals. Hurd-Karrer (1936) found that wheat plants grown on soil to which sodium selenate had been added at the rate of 30 p.p.m. Se contained 1,120 p.p.m. Se (air-dry basis) when young and but 220 p.p.m. at maturity. Similarly, from nutrient solutions containing 1 p.p.m. Se

TABLE 8

ABSORPTION OF SELENIUM BY VEGETABLES

Plant	Selenium added to soil		Selenium in resulting plant	
	Form	Amount	Part	Amount
		p.p.m.		p.p.m.
Carrots	Selocide	2	Tops	19
			Roots	7
	Selocide	20	Tops	25–43
			Roots	12
	Selenite	1.1	Tops	11–18
			Roots	2–4
Lettuce	Selocide	20	Tops	26
Peas	Selocide	20	Seeds	5–10
	Selenite	1.1	Seeds	10
Tomatoes	Selenite	1	Fruit	10
		10	Vines all died	
	Selenate	1	Fruit	28
		10	Vines all died	
	Selocide	1	Fruit	6
		10	Fruit	13
		100	Vines all died	

as sodium selenate, one-month-old wheat plants collected 330 p.p.m. Se, but at maturity the leaves contained 40 p.p.m., the stems 12 p.p.m., and the grain 8 p.p.m.

The various parts of a given species of plant may contain decidedly different concentrations of selenium. The data on wheat (preceding paragraph and p. 147) exemplify this condition. Analysis of a sample of *Astragalus pectinatus* when the seeds were beginning to disperse gave the following results (Beath, Eppson, and Gilbert, 1937):

Material	Selenium, p.p.m.
Pods	124
Foliage	260
Seeds	3,250

Combination in Which Selenium Occurs in Plants.—Several workers have attempted to obtain information regarding the manner in which selenium is held in plants by determining its solubility in water or other solvents. Unfortunately, there is poor agreement among the experimenters. Beath, Draize, *et al.* (1934) and Beath, Gilbert, and Eppson (1937) found the selenium in range plants of Wyoming to be freely water-soluble and about 50 per cent of that in wheat to be water-soluble. On the contrary, Horn, Nelson, and Jones (1936) by biological testing found that neither water, alcohol, nor ether removed selenium from wheat. Incidently, this proves that it cannot be associated with the lipoids. Moxon (1937) also, by a biological method, obtained no evidence that selenium was removed from corn by distilled water or by 5 per cent potassium sulfate solution.

The first suggestion that selenium may be present in plants as a result of replacing sulfur in some organic compound probably was made by Cameron (1880). Among more modern workers, Franke (1934) showed that the toxic material in Dakota wheat and corn followed the protein fraction. Pursuing this work further, Franke and Painter (1936) found little or none of the selenium to occur in the free state or in an inorganic compound. Even after hydrolysis of the proteins to amino acids, the selenium remained in organic combination. Upon extraction of the hydrolysate with chloroform, petroleum ether, alcohol, or benzene, none of the selenium was removed; but it all passed into butyl alcohol within 72 hours. This behavior is like that of the sulfur-containing amino acids cysteine, cystine, and methionine (Painter and Franke, 1935). All cystine precipitants brought down more or less of the selenium present. At least part of the total selenium is in an organic compound whose properties closely resemble those of cystine. The selenium:sulfur ratio in the protein from a sample of toxic wheat was found to be 1 :148. In general, the ideas of Franke and his co-workers were confirmed by Horn, Nelson, and Jones (1936). The selenium analog of cystine has been synthesized (Gordon, 1935), but apparently its biological effects have not been reported.

Probably selenium occurs in somewhat different forms in various plants; for Beath, Eppson, and Gilbert (1937) found that it is not volatile from cereals, grasses, or vegetables, but when the "selenium converters" are dried, up to two-thirds of their contained selenium may be lost. In fact, the living plants have an odor which is said to repel foraging animals. Byers and Knight (1935) reported that *Astragalus bisulcatus* can be freed almost completely from selenium by extraction with hot water, and that part of the selenium is volatile with steam. Franke and Painter (1938) showed that even "lethal wheat" becomes less toxic

as it is stored. Such behavior is probably due to loss of a volatile selenium compound.

Availability of Selenium to Plants.—Examination of the data of Byers (1935, 1936) indicates that there is no correlation between the concentration of selenium in various soils of the Middle West and Rocky Mountain regions and its concentration in any given species of plant grown on the soils. Since selenium occurs in several forms whose relative amounts doubtless differ in various soils, such a result is to be expected. Lakin, Williams, and Byers (1938) have reported that none of the naturally occurring vegetation from a certain district in Puerto Rico contains as much as 1.0 p.p.m. Se, although the soil content is from 2.5 to 12.0 p.p.m. Se. This situation has forced the above writers to the conclusion that, "Selenium content in soils does not necessarily indicate a dangerous or even slightly harmful situation." They also express the opinion that the selenium left in the soil as a result of the use of Selocide will be available to plants only as the result of slow hydrolytic processes which change it to available forms. In the absence of information concerning the fate of selenium in different combinations added to the soil, conclusions cannot be drawn regarding the true availability of selenium in its various states. The free element has but slight effect, if any, upon wheat (Hurd-Karrer, 1935) but upon a nonseleniferous soil to which free selenium was added to give 25 p.p.m., 3-months-old seedlings of certain *Astragalus* species contained 1,150 p.p.m. Se (Beath, Eppson, and Gilbert, 1937).

Wheat is able to take in much more selenium from added soluble selenate than from selenite (Hurd-Karrer, 1935). This may be the result of a true difference between the hexavalent and tetravalent forms of the element, but more likely it is a consequence of the greater reactivity of the selenite, which may be reduced to the free state or form very slightly soluble compounds—for example, with iron—and hence be less available to plants.

No systematic study of the effect of acid or basic conditions upon the absorption of selenium seems to have been made. Hurd-Karrer (1934, 1935) found no effect upon the toxicity of sodium selenate to wheat seedlings when the pH of soils varied from about 5 to 8. It does not follow that other forms of selenium would be similarly indifferent to pH.

The importance of the nature of the soil in absorption of added selenium by plants has been emphasized by Hurd-Karrer (1935). The physiological effects of sodium selenate upon wheat plants were much more decided in the case of Pierre clay than in that of Keyport clay loam. The latter soil has a relatively higher content of aluminum and iron and hence may be expected to react with or tightly adsorb more of the added

selenium compound. There is little possibility, however, that a soil containing dangerous amounts of selenium may be rendered suitable for use by adding iron, because the cheap iron compounds, such as commercial iron oxide, do not react to any appreciable degree with soluble selenium. The iron compounds which will form insoluble compounds with selenium—for example, iron hydroxide or soluble salts which hydrolyze to form it—are too expensive for agricultural use in the amounts needed. Addition of quartz sand to Keyport clay loam (Hurd-Karrer, 1935) increased the toxic effects upon wheat plants of a given concentration of sodium selenate in the mixture. Knight and Beath (1937) found that Thermopolis shales not only permit but little added selenite to be absorbed by wheat, but even the incorporation of highly seleniferous green *Astragalus bisulcatus* in the soil resulted in little selenium in the wheat grown thereon.

These same authors have reported an interesting difference between the distributions of added inorganic and organic selenium in several Wyoming shale soils. Sodium selenite tends to remain concentrated in the top layers, whereas that from seleniferous plants readily penetrates at least to the 2½-foot depth. That the growth of "selenium-converter plants" over long periods does not result in an accumulation of selenium near the top of the soil is shown by the tables of Byers (1935, 1936) and by the data of Beath, Eppson, and Gilbert (1937).

Effect of Adding Sulfur.—The close association of amino acids containing selenium with those containing sulfur has led to the suggestion that plants distinguish between them with difficulty. Hurd-Karrer (1936) has stated:

> The only way I have found so far to explain the relationship of sulfur and selenium is to assume that the root cannot tell the difference between them because of their chemical similarity. Assuming that this is true, then the amount of selenium taken in with a given amount of sulfur would depend on the proportionate amounts of the two which are available, the total absorbed being limited. Thus if there is a large excess of sulfur, the root will get relatively little selenium. After the selenium gets in, it can be assumed that the plant proceeds to use it as if it were sulfur, but with serious results. Every molecule that gets selenium instead of sulfur would be disabled, as it were, and fail to function normally. When a large enough proportion of the molecules are affected, the plant shows external signs of injury. This theory of substitution adequately accounts for the quantitative aspects of the dependence of selenium toxicity on relative rather than absolute sulfur availability; and for the fact that chemical analyses show that excess sulfur reduces the amount of selenium taken up by the plants.

In a series of reports, this same author (Hurd-Karrer, 1934, 1935; Hurd-Karrer and Kennedy, 1936) has cited numerous data which show

that the addition of sulfur in various forms to soils artificially selenized with sodium selenate decreases both the intake of selenium by plants (particularly wheat) and the toxic effects of the plants when fed to rats. Hurd-Karrer considers that a sulfur : selenium ratio of 12 : 1 or greater insures protection to plants grown on soils artificially selenized with sodium selenate although such wheat grain may be harmful to rats. As might be expected, free sulfur is effective only after time has been allowed for it to change into a soluble form.

Confirmation of the effect of soluble sulfur was obtained by Knight and Beath (1937) who raised wheat upon an originally nonseleniferous soil to which had been added several parts per million of selenium as sodium selenite. To one plot a mixture of sulfur and ammonium sulfate was added to the extent of 0.4 per cent (4,000 p.p.m.). The partly grown plants contained selenium as follows: on sulfur-treated soil, 32 p.p.m.; on untreated soil, 378 p.p.m.

The addition of hexavalent inorganic selenium compounds to soil does not duplicate its natural occurrence either in the original condition or after incorporation in a plant and subsequent return to the earth. Hence the effects of sulfur may be expected to depend upon the condition of the selenium. Hurd-Karrer (1935) found that free sulfur at 1 : 1,200 in a soil containing 4 p.p.m. of naturally occurring selenium reduced the selenium in plants (presumably wheat) grown on the soil from 450 p.p.m. Se to 15 p.p.m. Byers (1935) made the following cautious statement regarding the effects of sulfur on selenium absorption by range plants: "In general, where the soluble sulfates are high, i.e., the sulfur-selenium ratio is high, the selenium content of comparable samples is low, and, conversely when the sulfur-selenium ratio is low, the plant content is high. However, the relation is not consistent."

In contrast to these two reports, all other workers seem to have agreed that, under practical conditions, sulfur is of no value in decreasing selenium absorption. Martin (1936) reported that only low concentrations of sodium selenite—for example, 1 p.p.m. Se—in nutrient solutions could be rendered nontoxic to wheat plants by adding even large amounts of soluble sulfate. In fact, a S : Se ratio of 2.5 : 1.0 was as useful as one of 40 : 1. Franke and Painter (1937) pointed out that Hurd-Karrer's results are inexplicable to many workers familiar with the soils of some regions which produce seleniferous vegetation because these soils are nearly or completely saturated with calcium sulfate and the waters of the areas are rich in soluble sulfate. In their own experiments, no decrease in selenium absorption by corn, wheat, or barley was caused by 1,500 pounds per acre of ground sulfur or calcium sulfate added to a seleniferous soil

one and two years before. During the course of the present investigation, wheat was grown by Hoagland on culture solutions containing 8 p.p.m. Se as sodium selenite and varying concentrations of soluble sulfate. The mature grain was analyzed by Kirk with the following results:

S : Se ratio	Selenium in grain, p.p.m.
2:1	142
4:1	80
10:1	23.4
12:1	42.3

Obviously absorption of selenium was only partly prevented by a 12:1 sulfur : selenium ratio.

Knight and Beath (1937) and Moxon (1937) have arrived independently at the conclusion that sulfur does not inhibit the absorption of the organic combinations of selenium that are given to the soil when "selenium-converter" plants such as *Astragalus* decompose. This was still true when the seleniferous plant had been in the soil for three years. Beath, Eppson, and Gilbert (1937) cite data showing that young wheat plants took in *more* selenium from soil containing powdered *Astragalus* plants when sulfur, sodium sulfate, or magnesium sulfate was added. The foliage of beans grown on a soil plus 0.6 per cent S contained 150 p.p.m. Se, whereas on the same soil in the absence of sulfur, it contained 36 p.p.m. Se.

The large amount of data furnished by the workers mentioned immediately above discourages any hope that the addition of sulfur offers a method for mitigating the harmful effects of naturally occurring selenium in districts where they are serious. Even if the desired effects could be secured by generous use of sulfur, the cost would often be prohibitive.

TOXICITY OF SELENIUM TO ANIMALS AND MAN

The remarkable story of how a horse, cattle, and hog disease of certain states in the Great Plains and Rocky Mountain regions was traced to ingestion of selenium in the food has been told many times—for example, Trelease and Martin (1936), Steen (1936), Moxon (1937), and Knight (1937).

The distinction between the acute and chronic types of toxic action was recognized long ago. The term "blind staggers" has been applied to the sudden onset of violent symptoms such as impairment of vision, aimless wandering, gnawing of wood and metals, grating of teeth, drooling, grunting, labored breathing, and paralysis ending in death. This condition is the result of eating highly seleniferous plants such as the "selenium converters" and may come on very soon or after a considerable

delay (Beath, 1935; Knight and Beath, 1937). Ordinarily, animals avoid these plants, but in time of drought may be driven to eat them, and animals newly arrived from other localities are not careful to avoid them at any time.

"Alkali disease" signifies the chronic condition characterized by retarded growth of the young, dullness, apathy, emaciation, loss of hair, aged appearance, soreness of joints, and loosening or deformed growth of hoofs. This is the result of continued ingestion of small amounts of selenium, particularly as it occurs in cereals and forage crops.

A detailed summary of the pathologic anatomical changes in both conditions as observed at autopsy has been given by Draize and Beath (1935) and by Smith, Stohlman, and Lillie (1937). Selenium is present in all parts of the body to the extent of several parts per million, especially in the liver, kidney, and spleen (Dudley, 1936). Degenerative changes, such as fatty infiltration and necrosis of the liver and advanced anemia, are more pronounced, of course, in the condition of chronic poisoning.

Proof that Selenium Is the Cause of the Disorders.—After suspicion was first aroused that selenium might be the cause of blind staggers and alkali disease, it was necessary to determine if the incidence of the disease corresponded geographically with the occurrence of selenium in plants used for animal food, and to compare the effects of selenium introduced into the body in various forms and ways with the natural symptoms. A review of the very extensive research work which has elucidated these questions is not necessary here, for it has been discussed at length by several writers. Byers (1935), Franke, Rice, *et al.*, (1934), and Knight (1935) have shown convincingly that the area in which selenium occurs to the extent of at least several parts per million in the food of livestock is the same as that in which the disorders occur. Differences between regions are to be expected—for example, alkali disease is the predominant type in South Dakota (Moxon, 1937) where grain is raised very extensively, whereas blind staggers is typical of the range lands of Wyoming.

The attempts to prove that the naturally occurring symptoms are identical with those resulting from experimental feeding of selenium and hence may be attributed solely to that element have resulted in general agreement, although points of difference still persist among various workers. For one thing, experiments have been made with rats, cats, and other small animals, whereas practical interest is in large animals and humans. Beath has written (Knight and Beath, 1937):

The writer has no report of anyone who has produced aggravated cases of the alkali disease in cattle and horses by experimentally controlled feeding tests. Nor has any investigator reported upon the production of the acute stage of blind staggers by

feeding, under controlled conditions, those seleniferous weeds believed to be responsible.... So far as the writer can determine, no one has as yet shown that an aggravated case of the alkali disease characterized by deformed hoofs, sloughing of manes and tails, and other loss of hair, can be produced in cattle and horses by daily feeding of the inorganic salts of selenium.

It has been suggested (Beath, Eppson, and Gilbert, 1935) but not proved that other elements, such as tellurium or molybdenum may be concerned in these disorders.

With small experimental animals, the picture is much clearer. On the criteria of growth, food intake, hemoglobin level, and gross pathology of rats, Franke and Potter (1935) decided that the effects of sodium selenite added to a standard diet are "virtually identical" with those produced by the natural plant toxicant, and that for equal intakes of selenium, the toxic effects are quantitatively the same. Schneider (1936) also found that selenite produced the same symptoms in his rats as Franke had described. Martin (1936) compared the effects upon rats of sodium selenite added to a standard Sherman and Campbell diet with those of seleniferous buckweat and found the toxicities to be identical. The identity of symptoms was corroborated by Munsell, De Vaney, and Kennedy (1936), but these workers found the condition of animals on the diet containing 18.4 p.p.m. selenite selenium to resemble most nearly that of others on toxic wheat diet containing 9.8 p.p.m. Se, that is, the naturally occurring selenium was about twice as toxic as the inorganic form.

In an elaborate summary of nearly ten years' work with rats, Franke and Painter (1938) have declared that on both naturally and artificially selenized diets, the toxic effects are due solely to the presence of selenium. As criteria they chose: average life span, average net gain, difference in net gain between control and experimental, average food consumption and difference in food consumption of control and experimental. For the different sources of the element, the order of decreasing toxicity for diets containing 10 p.p.m. Se or more was wheat, corn, barley, selenite. However, the differences are not great, for example, equal toxicity is associated with the following selenium content in the diets: wheat, 14 p.p.m.; corn, 16 p.p.m.; barley, 18 p.p.m.; selenite, 25 p.p.m. Any difference in toxicity between naturally occurring selenium compounds in plants and selenite is probably due to reduction of the latter to free selenium, which is very poorly absorbed.

Restriction of Food.—All workers have noted that rats are loath to eat food containing selenium. Franke and Potter (1936*a*) found that they invariably chose the food containing the least selenium whether it was present in wheat or as sodium selenite. In the experiments reported by

Miller and Schoening (1938), young pigs were very loath to eat food containing 24.5 p.p.m. Se as sodium selenite. This is entirely analogous to the previously mentioned tendency of animals on the range to avoid seleniferous plants. The question of whether the effects of a diet containing selenium may be due at least in part to the voluntary partial starvation of the test animals has received considerable attention. Symptoms such as diminished growth of the young, loss of weight of the adults, weakness, and general apathy are the usual results of starvation; but the degenerative changes which persist after animals are restored to normal diets are clearly specific results of the ingestion of selenium. Restriction of a normal diet to equal the amount of a seleniferous diet eaten resulted in about equally diminished rates of growth; but the hemorrhages, degenerate liver, and other pathological symptoms were entirely limited to the rats on a diet containing selenium (Munsell, De Vaney, and Kennedy, 1936). The whole matter was examined critically by Franke and Painter (1938) who concluded that: (*a*) although there is a high correlation between the selenium content of the diet and voluntary food restriction, there is no correlation between the toxic effects produced and the daily consumption of selenium; (*b*) when much selenium is in the diet, the food intake is reduced to a level which will not maintain life and enable the animal to withstand the toxic action of the selenium; (*c*) on the diets which caused a diminution in food intake, the rats eating the most selenium fare the best; (*d*) animals on a seleniferous diet gain less weight per gram of consumed food than they do on normal diets. If this last conclusion holds also for farm animals, tremendous economic losses may be suffered by stockmen in the seleniferous regions entirely aside from the direct losses by illness and death of animals.

Effects on Reproduction and the Survival of Young Animals.—A further source of losses of the less obvious kind is that of decreased fertility as a consequence of ingesting selenium. It has long been known that in certain localities of South Dakota unsatisfactory hatching of eggs and malformation and high mortality of chicks are of general occurrence. In a series of studies, Franke and his co-workers (Franke and Tulley, 1935, 1936; Tully and Franke, 1935; Poley, Moxon, and Franke, 1937) showed that these effects are the result of selenium in the chicken feed, and that similar effects may be produced by injecting selenite into the air cell of eggs before incubation. In a feeding experiment in which chickens were placed upon a diet containing 65 per cent seleniferous grain (15 p.p.m. Se), after 7 days no more normal chicks were produced as long as the diet was continued, and the hatchability was reduced to zero in 13 to 19 days; 6 days after removal from the toxic diet these symptoms disappeared.

TABLE 9

DATA ON ACUTE TOXICITY OF SELENIUM AND OF ARSENIC

Animal	Workers	Method of administering	Form of chemical	Minimum fatal dosage, mg per kg of body weight	Remarks
		Selenium			
White rat	Muehlberger and Schrenk (1928)	Intravenous	Selenite Selenate	4.7 4.3	
	Jones (1909)	Hypodermic	Selenite	3.2	Assuming weight of rat 125 grams
	Smith, Franke, and Westfall (1936) Smith, Stohlman, and Lillie (1937)	Intravenous	Selenite Selenate	3.0 3.0	Selenite acts more slowly
	Franke and Moxon (1936)	Intraperitoneal	Selenite Selenate	3.25–3.5 5.25–5.7	
Rabbit	Muehlberger and Schrenk (1928)	Intravenous	Selenite Selenate	0.9 1.1	
	Smith, Stohlman, and Lillie (1937)	Intravenous	Selenite Selenate	1.5 2.5	
Cat	Smith, Stohlman, and Lillie (1937)	Subcutaneous	Selenite	2 – 3	Causes vomiting
Dog	Woodruff and Gies (1902)	Oral, hypodermic or rectal	Selenite or Selenate Powdered Se	4 4	Fatal in a few minutes 6 grams by mouth had no effect
		Arsenic			
White rat	Franke and Moxon (1936)	Intraperitoneal	Arsenite Arsenate	4.25–4.75 14.0–18.0	
	Smith, Franke, and Westfall (1936)	Intravenous	Arsenite	5 – 6	
	Muehlberger and Schrenk (1928)	Intravenous	Arsenite Arsenate	6.0 21.0	
Rabbit	Muehlberger and Schrenk (1928)	Intravenous	Arsenite Arsenate	6.0 7.0	
Man	McNally (1937)	Oral	Arsenite	2.5–3.0	

In a study with rats on diets containing seleniferous wheat, Franke and Potter (1936*b*) showed that prolonged feeding led to a distinct loss in reproductive power. Matings between animals both fed on the toxic grain were invariably sterile; if but one animal had been on the diet, fertility was variable.

Affected females could not raise their young. There is a strong probability that failure of the young to survive was at least partly due to their susceptibility to selenium poisoning as well as to any failure of the mothers to give proper care, for it has been found that selenium occurs to at least 1 p.p.m. in the milk of cows on a seleniferous diet (Smith and Westfall, 1937), and doubtless is present in rat milk under the same circumstances. The hypersensitivity of the young to selenium poisoning is illustrated by the results of Franke and Potter (1936*b*), who found that young rats aged 20–22 days put on a diet of wheat containing 24.6 p.p.m. Se died in a few weeks, whereas those not exposed to the diet until they were 60–100 days old lived through a feeding period of 360 days with no outward signs of injury except a somewhat depressed rate of growth.

There is no proof that similar conditions hold for larger animals, but this work suggests that this and other aspects of chronic selenium ingestion may be of practical significance.

Quantitative Data on Toxic Effects Due to Selenium.—Like many other substances which are harmful to animals, the effects caused by selenium vary greatly with such factors as size of dosage, form and manner of administering, and varying susceptibility of different test animals. Distinction between the acute, subacute, and chronic symptoms and dosages is important. It is not always possible to ascertain at what age the animals were used, but, in general, in experiments not involving growth, young adults were used, whereas growth studies usually were started soon after weaning.

The data on acute toxicity refer to the situation in which death follows a single dose within a very few days at the latest. Part of the pertinent data are summarized in table 9. For comparison, certain data on the acute toxicity of arsenic are included.

When death usually ensues but is delayed for a considerable time, the toxicity may be referred to as "subacute." The data in table 10 are for animals kept on a seleniferous diet continuously. In some cases, food consumption has not been reported, and hence no relation between intake of selenium and toxicity can be examined. It will be noted that 1.5 mg Se per kg of body weight daily results in the death of about 30 per cent of the rats after several months on the diet, whereas rabbits and cats are affected at least as severely by 0.3 mg Se per kg of body weight daily or

TABLE 10

DATA ON SUBACUTE TOXICITY OF SELENIUM

Animal	Workers	Source of selenium	Selenium concentration in diet	Daily dosage per kg body weight	Remarks
			p.p.m.	*mg*	
White rat	Nelson, Hurd-Karrer, and Robinson (1933)	Wheat	8-10	..	Death in a few weeks
White rat	Franke and Painter (1938)	Corn			*Per cent dead in 70 days:*
			9.9		40
			10.7		25
			12.2		10
			13.1		30
			16.4		70
			17.5		50
			17.5		40
			20.5		100
			23.0		100
White rat	Martin (1936)	Selenite or buckwheat	22		Majority died in 42 days
			64		All died in 5-7 days
White rat	Trelease and Trelease (1937)	Wheat	65		All died in 4-11 days
White rat	Smith, Stohlman, and Lillie (1937)	Selenite	15	1.5	29 per cent died in 30-172 days
		Selenate	15	1.5	None died in 72-117 days
Rabbit	Smith, Stohlman, and Lillie (1937)	Selenite		0.3	6 in 9 died after 17-99 doses, 3 survived 113 doses
Cat	Smith, Stohlman, and Lillie (1937)	Selenite		0.25	2 in 5 died after 60-96 doses, 3 survived 122-150 doses
				0.1	1 in 4 died after 40 doses, 3 survived 140 doses
Pig (4 months old)	Miller and Schoening (1938)	Selenite	24.5	0.40 (av.)	Died in 79 days
				0.87 (av.)	Died in 46 days

less. On the basis of results with only a few animals, the susceptibility of young pigs to subacute poisoning from selenium appears to be between the susceptibilities of white rats and of rabbits and cats.

From the standpoint of public health, the chief consideration in the use of selenium in a spray is the possibility of causing chronic effects which may never result in death but nevertheless may impair general health and efficiency. The data for such conditions, as given by various workers, refer both to selenium content in the food and to actual amounts of selenium ingested. Barley hay containing 6 p.p.m. Se fed to cattle for several months and *Atriplex Nuttallii* (19 p.p.m. Se) fed to cattle and to hogs for 3 months caused no sign of injury (Beath, Eppson, and Gilbert, 1935). Rats on diets containing 1.5 p.p.m. Se in wheat grew normally, reproduced, and reared young fully as successfully as those on the standard diet; with 3 p.p.m. Se in wheat, growth was normal, but reproduction was slightly decreased (Munsell, De Vaney, and Kennedy, 1936). Munsell, De Vaney, and Kennedy (1936) found the addition of selenious acid to the extent of 9.1 p.p.m. Se in the diet of rats caused no abnormalities upon autopsy after 38 weeks. Sodium selenate in the diet at the rate of 7.5 p.p.m. Se did not depress growth of rats, and no pathological symptoms were observable after 6 months (Smith, Stohlman, and Lillie, 1937). As a result of their extensive experience, Franke and Painter (1938) state that any diet containing over 5 p.p.m Se will retard the growth of rats and one with over 9 p.p.m. will kill rats placed upon it soon after weaning. In experiments conducted by C. D. Leake, of the Pharmacology Division, wheat containing 23.4 p.p.m. Se was found to cause no mortality in young rats within 15 days, but a delay in resuming normal growth persisted for several weeks after the normal diet was restored. This probably indicates that more or less permanent injury had been produced. Probably the conclusion of Byers (1935, 1936), that the tolerance limit is 3–4 p.p.m. Se in all the diet, is a fair summary of the results to date.

The data relating known daily intake of selenium with chronic effects are very scanty. From the information given by Martin (1936), it may be calculated that on a diet containing 12.5 p.p.m. Se in buckwheat the average daily intake of rats was 1.35 mg Se per kg of body weight. Normal growth was obtained on this diet; but when 17.5 p.p.m. Se was present and the daily intake was 1.63 mg Se per kg of body weight, the growth was slightly retarded. In similar experiments with diets containing seleniferous wheat, Munsell, De Vaney, and Kennedy (1936) found the weekly intake of 0.253 mg Se per 100 grams of body weight (0.361 mg Se per kg daily) to result in retarded growth and failure to reproduce. No obvious explanation for the difference in these results is at hand.

Excretion of Selenium.—Another method is available for calculating the amounts of selenium ingested by animals, and it is applicable also to humans. Munsell, De Vaney, and Kennedy (1936) found that the amount of selenium stored in a rat's body does not increase indefinitely but reaches the maximum for a given diet in 4 to 8 weeks. Thereafter a balance is maintained if the intake is constant and the animal is not progressively injured. Selenium is excreted in at least three ways—by the urine, feces, and breath. Analyses have shown that the urinary excretion accounts for most of the total loss, for example, 50–80 per cent in the cat (Smith, Westfall, and Stohlman, 1937). Hence, it may be used to calculate a minimum figure for the selenium intake with the probability that the true intake will be higher but not more than twice as great. Dudley (1936) found from 0.1 to 3.0 p.p.m. Se in the urine of hogs which later died as a result of ingesting 15.6 to 74.4 mg Se per kg of body weight daily. A horse was fed 94.0 grams Se as sodium selenite over a period of a year's time; near the end of the feeding period, a sample of urine contained 2.0 p.p.m. selenium; no ill effects of the selenium diet were observed. The data given by Smith, Franke, and Westfall (1936) for urine from several horses suffering from alkali disease are particularly interesting: a colt at autopsy, 0.33 p.p.m.; horse No. 1, 1.00 p.p.m.; horse No. 2, 1.25 p.p.m.; horse No. 3, 1.70 p.p.m.

Valuable information regarding the elimination of selenium after its ingestion has ceased is given by urine analysis. Smith, Westfall, and Stohlman (1937) found that the bulk of stored selenium was eliminated by cats within 2 weeks, but that small amounts occurred in the urine for at least a month longer. Munsell, De Vaney, and Kennedy (1936), by analysis of rat tissues at various times after removal of selenium from the diet, found somewhat comparable conditions with the rat, though depletion was not attained for a longer period.

Smith, Westfall, and Stohlman (1938) have published data on the relative ease of excretion of inorganic and organic selenium by cats and rabbits. Their results show that over feeding periods up to 144 days less than half the ingested selenium from wheat gluten or oats was excreted in the urine. The amounts of selenium stored in the liver and other organs were correspondingly greater than when inorganic selenium was fed. These results were obtained with diets relatively rich in selenium, however, and give no information on the effects of extremely small intake of organic selenium over long periods.

Chronic Effects of Selenium upon Humans.—The most important application of the study of urinary secretion of selenium is to the case of humans who live in the regions in which the vegetation is known to be

seleniferous. Smith and co-workers have made two surveys of families in the seleniferous regions of South Dakota, Nebraska, and Wyoming. In the first (Smith, Franke, and Westfall, 1936) the results for 127 individuals in 90 families may be summarized:

Selenium in urine, p.p.m.	Number of persons	Per cent of total
0	4	3.1
trace	6	4.8
0.02–0.09	35	27.6
0.10–0.19	22	17.3
0.20–0.49	37	29.2
0.50–0.99	19	14.9
1.00–1.33	4	3.1
TOTAL	127	100.0

The second survey (Smith and Westfall, 1937) covered 100 individuals (including several of the first survey) and showed the urinary selenium to vary from 0.20 to 1.98 p.p.m. Se. The authors have calculated that the persons examined have been absorbing continuously from 0.01 to 0.10, or possibly 0.20 mg Se per kg of body weight per day. This indicates a daily absorption of 1 to 2 mg, and in some cases possibly 5 mg, of selenium for an adult. Since these people had been residing in the same localities for from three to forty years, they were presumably at an equilibrium condition. The conclusion from physical examinations of the subjects was: "Outside of a high incidence of symptoms pointing to gastric or intestinal dysfunction, and a few instances of apparent hepatic dysfunction, both probably the result of continual selenium ingestion, no other evidence of ill health was seen that could be ascribed to selenium with any degree of certainty."

To gain information regarding the sources of the selenium to which persons included in these surveys were exposed, analyses were made of several foods. The results in brief were:

Food	Number of samples	Selenium, p.p.m.
Canned or salted pork	4	1.17 to 8.00
Raw chicken muscle	1	2.19
Lean cooked beef	1	2.22
Milk	50	0.16 to 1.27
Eggs	32	0.25 to 9.14
Grains	42	0.87 to 18.80
Vegetables	95	0.00 to 17.80

A study of the relation between selenium content of the various foodstuffs and urinary elimination proved that the chief sources are meats, eggs, milk, and grains. Vegetables played little part, but since they were

scarce during the drought year of 1936, this conclusion may not be true in general.

For comparison, the data given by Byers (1935) and by Dudley and Byers (1935) show from 0.02 to 3.00 p.p.m. Se in whole milk from the same general region and 9.6 p.p.m. Se in the milk solids.

DISCUSSION AND CONCLUSIONS

The attention of the medical profession, of scientific and popular writers, of governmental agencies, and of the general public has been aroused during recent years to the health hazard arising from the use of various poisonous substances for the protection of plants which are used as foods. Entomologists and agricultural chemists are well aware of the situation, and a truly enormous effort is being made to eliminate such toxic elements as lead, arsenic, etc., from agricultural use and to substitute organic substances in their place. As yet, however, no more than a beginning has been made. In the face of this feeling against inorganic materials, the advisability of using such a product must be considered with particular care. If it offers a menace to public health, its use should be abandoned if possible and carefully controlled in any event. The two criteria are: how poisonous is the substance in question and how much of it will be present in food as a result of its use for control of pests? The foregoing lengthy account of work in this and other laboratories is intended to be a summary of the pertinent available information from which conclusions may be drawn regarding the use of the selenium-containing product Selocide, particularly on citrus trees and grapevines.

There is almost no conceivable danger of acute poisoning from eating citrus or grapes treated with Selocide, for it may be estimated from the data given that the amount of selenium needed is several hundred milligrams, which would be furnished by half a ton or more of oranges or grapes. The real question is concerned with the cumulative effect of daily ingestion of small amounts of selenium. The toxicity data indicate that for several animals the safe limit, that is, the amount which produces no detectable chronic symptoms, is about 3 p.p.m. Se in the whole diet. It appears that this figure may be used for humans also since the food of the families in the seleniferous regions contained at least this much selenium on the average and no definite symptoms attributable with certainty to selenium were found in the group. There is no decided difference in the toxicity of inorganic and organic selenium, except that the free element has very little effect, owing in part to restricted absorption from the digestive tract. If the use of Selocide resulted in the high concentration of 0.2 p.p.m. Se in the fruit and if it is all absorbed during digestion,

a simple calculation shows that in order to ingest 1 mg of selenium per day, a person must eat about 11 pounds of the contaminated product daily. The results of Smith, Westfall, and Stohlman (1937) indicate that 1 mg of selenium per day is not harmful to an adult. Even though children, like young rats, probably are more sensitive to selenium than adults, a very large amount of contaminated fruit would be necessary to exert a harmful effect. Of course, if selenium is present in other constituents of the diet, an additional intake from any source may cause the total intake to reach or exceed the safe limit, but in that case, the rest of the diet usually is the greater source of danger. The special case of by-products in which selenium might become concentrated is a matter which the industries concerned must attend with especial care, just as also must be done in the cases in which lead or arsenic are involved.

The subacute and chronic effects of selenium upon lower animals have been shown to be due in part to voluntary decrease in intake of seleniferous food. In the case of human diets, only parts of which contain appreciable amounts of selenium, it is reasonable to expect this effect of semistarvation to be diminished or eliminated. Hence, under conditions of choice of food, the results of daily ingesting a given amount of selenium should be less than when all the food is seleniferous. It does not seem likely that the amounts of selenium which may occur in human foods would lead to avoidance of such foods, for Franke and Potter (1936*a*) found that rats made but little distinction between control grain and that containing 6.15 p.p.m. Se, though the young ones were very reluctant to eat grain containing 12.3 p.p.m. Se.

In order to remove the uncertainty of agriculturists regarding possible legal complications from the use of selenium, it is desirable that a tolerance be set. The indications are that 3 p.p.m. Se would be entirely safe, but a lower figure would probably work no hardship and would be a stronger guarantee to the public that their food was fit to eat.

Although selenium occurs in large amounts in only a few plant species, the indication is that all plants take it up to some extent. Hence the element is not a stranger to the diet of animals or man, and the intake required to produce toxic effects is probably the result of a physiological tolerance acquired by exposure through the ages. There seems to be no reason to hope that this natural partial immunity to selenium can be increased rapidly: Smith, Stohlman, and Lillie (1937) found no evidence of such an effect with cats.

The analyses discussed in a previous section show that the amount of selenium added to a diet as the result of its use on citrus is extremely minute. The residue on the outside is in the least toxic (elementary) form

and ordinarily would not be eaten. Furthermore, nearly all of it is removed during the regular packing-house treatment. Most of that which occurs in the interior of the fruit will ordinarily be ingested, though there is some evidence that in other plants the seeds contain more selenium than the other parts. Inspection of the data shows that over a period of five years' use there is only a very minute increase in the amount of selenium in the interior of citrus fruits. This is to be expected from the behavior of the selenium which reaches the soil as the result of the spraying process, for most of it remains in the first 6 inches and practically none has reached beyond the first 2 feet. Doubtless longer use will eventually result in deeper penetration so that more selenium will reach the feeding zone of citrus roots. Whether or not this will result in a considerable increase in absorption depends to a large extent upon the rainfall and irrigation practice.

Rainfall in the citrus districts of California varies considerably but may be taken as 15 inches on the average. Irrigation adds many more inches of water each year in most of the citrus districts. However, a considerable part of the irrigation water used up to the present time is obtained from wells in the various districts and hence is recirculated water. This condition is not so favorable for removal of soluble selenium as prevails in localities such as the Belle Fourche district of South Dakota (Byers, 1935), in which much of the water leaches through the soil and flows away by gravity. The introduction of irrigation water from the outside would increase the probability that soluble selenium will remain at a low level in the soil of the citrus district in southern California.

In the case of grapes, conditions are not so favorable for the use of selenium sprays. In the first place, the residue is greater and there is less chance to remove it, particularly from fresh fruit. Proper washing before drying should keep the selenium residue on raisins down to a low figure. The assimilation of selenium from the soil by grapes appears to be somewhat greater than in the case of citrus, but the analyses have all been made on grapes from the same district and conditions may be different elsewhere. Another factor to be considered is the probability that vineyards will be torn out in the course of time and the land used for other crops. Again in this case, the amount of water received by the soil is a very important factor; probably the normal rainfall or rainfall together with necessary irrigation is sufficient to prevent any serious accumulation of selenium as the result of the proper use of Selocide.

SUMMARY

A spray made by dissolving selenium in a solution of potassium ammonium sulfide in such proportions that the composition corresponds to the empirical formula $(K\,NH_4S)_5Se$ is effective for the control of mites on citrus and grapes. After several years' use, there is no evidence of harmful effects upon the plants, and fruit injury has occurred to only a limited extent in certain districts.

Analyses of citrus fruits has shown that the residue, which is mainly free selenium, is present to the extent of two to practically zero parts per million *in the rind,* according to the length of time between the application of the spray and the analysis. Analyses of the soil beneath sprayed trees showed that, after five years' treatment, a maximum of two parts per million selenium occurred in the first 6 inches, but penetration to lower depths was very slow. Absorption from the soil resulted in only a few hundredths part per million selenium in the interior of citrus fruits.

The residue upon grapes is somewhat greater on account of the larger surface which they present to the spray, and the analytical data indicate that they absorb somewhat more selenium from the soil.

The data regarding the acute, subacute, and chronic toxic effects of ingested selenium have been reviewed. There is considerable evidence that three parts per million selenium in the whole diet causes no symptoms of injury. Such an amount could not be obtained from citrus fruit or grapes. Hence it is concluded that proper use of the selenium-containing spray mentioned above on citrus and grapes, under the prevalent conditions of production in California, offers no hazard to public health.

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TOXICITY STUDIES WITH ARSENIC IN EIGHTY CALIFORNIA SOILS[1, 2]

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INTRODUCTION

THE INCREASING USE of arsenic in herbicides, insecticides, and soil sterilants presents problems of great economic importance. The farmer, needing practical methods for controlling pests, seeks the cheapest and most effective reagents, whereas the soils investigator must try to conserve our agricultural areas for present and future generations.

Arsenic, being cheap, readily available, and extremely toxic, is in constant demand for weed and insect-pest control and is recommended by many companies, often without specific knowledge of dosages required, effective methods of application, or ultimate effects upon the soil.

In the field use of arsenic, workers naturally ask what form is most effective for the particular type of treatment being used, how much will be needed for the desired results, and how long the results will last. The soils investigator wants to know what the effects of long-time accumulation of arsenicals in soils will be, whether the soil is permanently harmed when crop yields have been reduced, and how one may remove or remedy the toxic condition resulting from arsenic in the soil.

A previous publication (*6*)[5] presented data on arsenic toxicity[6] in four

[1] Received for publication January 17, 1938.

[2] This paper was made possible by the coöperative project on control of noxious weeds conducted by the California Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

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[5] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

[6] The term "toxicity" has acquired a wide variety of meanings. For purposes of the present group of papers (*7, 8, 13*) the criterion adopted is the application of chemical causing an almost complete suppression of growth. This use of the word has developed because in the control of weeds the practical object is to inhibit development completely.

TABLE 1

CHARACTERISTICS OF CALIFORNIA SOILS USED IN TOXICITY SERIES*
(All samples from surface 4 inches)

No.	Soil type	Origin	Mode of formation	Stage of development	Color	Remarks†	Moisture content of cultures	Soil used in each culture
				Sands				
							per cent	gm
1	Holland loamy gravelly sand	Acid igneous	Primary	Immature	Brown	Slightly acid	12.8	500
2	Niland gravelly sand	Mixed	Secondary alluvial	Youthful	Light brown-gray	Calcareous; saline	10.0	500
3	Oakley sand	Mixed	Secondary aeolian	Youthful	Light brown	Slightly acid	6.5	600
4	Rositas fine sand	Mixed	Secondary alluvial	Recent	Light brown-gray	Alkali; calcareous	14.5	500
5	Superstition gravelly sand	Mixed	Secondary alluvial	Youthful	Light gray	Calcareous	12.4	500
6	Tujunga sand	Acid igneous	Secondary alluvial	Recent	Light brown-gray	Neutral	12.3	500
				Gravelly and sandy loams				
							per cent	gm
7	Aiken gravelly loam	Basic igneous	Primary	Semimature	Red	Slightly acid	13.3	500
8	Arbuckle gravelly sandy loam	Sedimentary	Secondary alluvial	Youthful	Brown	Neutral	10.0	500
9	Chualar fine sandy loam	Acid igneous	Secondary alluvial	Immature	Dark brown	Neutral	13.0	600
10	Columbia fine sandy loam	Mixed	Secondary alluvial	Recent	Light gray-brown	Neutral	18.0	500
11	Corning gravelly loam	Mixed	Secondary alluvial	Semimature	Brown-red	Moderately acid	12.2	500
12	Delano fine sandy loam	Acid igneous	Secondary alluvial	Immature	Light red-brown	Basic	15.0	600
13	Foster fine sandy loam	Acid igneous	Secondary alluvial	Recent	Dark brown-gray	Neutral	16.2	500
14	Fresno sandy loam‡	Acid igneous	Secondary alluvial	Mature	Brown-gray	Alkaline	15.0	600
15	Greenfield coarse sandy loam	Acid igneous	Secondary alluvial	Youthful	Brown	Neutral	15.0	600
16	Hanford sandy loam	Acid igneous	Secondary alluvial	Recent	Light brown	Neutral	14.0	500
17	Hanford fine sandy loam	Acid igneous	Secondary alluvial	Recent	Light brown	Neutral	13.8	500
18	Meloland fine sandy loam	Mixed	Secondary alluvial	Recent	Light gray-brown	Alkali; calcareous	18.3	500
19	Merced fine sandy loam	Acid igneous	Secondary alluvial	Semimature	Dark gray	Neutral	15.6	500
20	Oakdale coarse sandy loam	Acid igneous	Secondary alluvial	Recent	Gray-brown	Neutral	10.8	600
21	Ramona sandy loam	Acid igneous	Secondary alluvial	Immature	Brown	Neutral	10.7	500
22	Redding gravelly loam	Mixed	Secondary alluvial	Mature	Brown-red	Moderately acid	13.3	500
23	Rocklin sandy loam	Acid igneous	Secondary alluvial	Mature	Brown-red	Slightly acid	17.9	500
24	Salinas fine sandy loam	Mixed	Secondary alluvial	Immature	Dark brown-gray	Neutral	14.4	500
25	Sierra gravelly loam	Acid igneous	Primary	Semimature	Brown-red	Moderately acid	12.5	500
26	Sierra sandy loam	Acid igneous	Primary	Semimature	Brown-red	Moderately acid	13.5	500
27	Sites fine sandy loam	Sedimentary	Primary	Semimature	Brown-red	Moderately acid	12.0	500
28	Tulare fine sandy loam	Mixed	Secondary alluvial	Immature	Light gray	Calcareous	20.0	500
29	Yolo fine sandy loam	Sedimentary	Secondary alluvial	Recent	Brown	Neutral	15.0	500

							per cent	gm
	Loams							
30	Egbert loam.	Mixed organic	Secondary alluvial	Immature	Dark gray	Slightly acid	32.0	500
31	Farwell loam. .	Basic igneous	Secondary alluvial	Youthful	Chocolate-brown	Neutral	17.6	500
32	Gridley loam.	Basic igneous	Secondary alluvial	Semimature	Brown	Slightly acid	22.1	500
33	Honcut loam.	Basic igneous	Secondary alluvial	Recent	Red-brown	Slightly acid	21.4	500
34	Madera loam	Acid igneous	Secondary alluvial	Mature	Brown	Neutral	12.0	500
35	Panoche light loam...	Sedimentary	Secondary alluvial	Recent	Brown-gray	Calcareous	14.0	500
36	Pinole loam .	Sedimentary	Secondary alluvial	Immature	Yellow-brown	Slightly acid	15.0	500
37	Placentia light loam..	Acid igneous	Secondary alluvial	Semimature	Brown-red	Neutral	10.4	500
38	Pleasanton loam	Sedimentary	Secondary alluvial	Immature	Brown	Neutral	16.7	500
39	Pond heavy loam	Acid igneous	Secondary alluvial	Immature	Brown-gray	Alkaline; calcareous	11.7	500
40	San Joaquin loam	Acid igneous	Secondary alluvial	Mature	Brown-red	Moderately acid	21.0	500
41	Tehama loam	Mixed	Secondary alluvial	Immature	Light yellow-brown	Neutral	13.6	500
42	Vina loam...	Basic igneous	Secondary alluvial	Recent	Brown	Neutral	22.5	500
43	Yolo loam ..	Sedimentary	Secondary alluvial	Recent	Brown	Neutral	20.1	500
	Silt and clay loams						per cent	gm
44	Aiken clay loam . .	Basic igneous	Primary	Semimature	Red	Slightly acid	21 3	400
45	Antioch clay loam .	Sedimentary	Secondary alluvial	Mature	Dark brown	Moderately acid	15.8	600
46	Arbuckle clay loam...	Sedimentary	Secondary alluvial	Recent	Brown	Neutral	18.5	500
47	Chino silty clay loam...	Acid igneous	Secondary alluvial	Youthful	Dark gray	Neutral	20.4	500
48	Columbia silty clay loam..	Mixed	Secondary alluvial	Recent	Light gray-brown	Neutral	26.0	500
49	Mariposa silt loam..	Sedimentary	Primary	Semimature	Brown-yellow	Moderately acid	20.0	500
50	Marvin silty clay loam	Mixed	Secondary alluvial	Immature	Light brown	Neutral	24.4	500
51	Ramada silt loam .	Mixed	Secondary alluvial	Recent	Light yellow-brown	Neutral	19.7	500
52	Sacramento clay loam	Mixed	Secondary alluvial	Immature	Dark gray	Slightly acid	28.2	400
53	Yolo silt loam. ..	Sedimentary	Secondary alluvial	Recent	Brown	Neutral	18.1	500
54a	Yolo clay loam§..	Sedimentary	Secondary alluvial	Recent	Brown	Neutral	30.0	500
54b	Yolo clay loam§	Sedimentary	Secondary alluvial	Recent	Brown	Neutral	30.0	500

* Information from (*14*, *15*, and *16*).

† Data given in this column were taken from (*14*, *15*, and *16*). They apply to the soil types in general. No such determinations were made on the samples collected.

‡ The Fresno sandy loam used in this and in previous tests is designated as brown phase. Shaw now classifies this soil in the Dinuba series (*14*).

§ Soil 54*a* harvested December 22, 1934; soil 54*b* harvested June 4, 1935.

(Table concluded on next page.)

TABLE 1—(*Concluded*)

No.	Soil type	Origin	Mode of formation	Stage of development	Color	Remarks†	Moisture content of cultures	Soil used in each culture
				Clays				
							per cent	*gm*
55	Alamo adobe clay	Mixed	Secondary alluvial	Mature	Dark gray	Neutral	27.3	500
56	Anita adobe clay	Basic igneous	Secondary alluvial	Immature	Dark brown	Neutral	26.3	500
57	Capay adobe clay	Sedimentary	Secondary alluvial	Immature	Gray-brown	Neutral	27.9	500
58	Clear Lake adobe clay	Sedimentary	Secondary alluvial	Youthful	Gray-black	Neutral	24.4	500
59	Conejo adobe clay	Basic igneous	Secondary alluvial	Recent	Gray-black	Neutral	29.2	500
60	Diablo adobe clay	Sedimentary	Primary	Semimature	Gray-black	Neutral	23.1	500
61	Dublin adobe clay	Sedimentary	Secondary alluvial	Recent	Gray-black	Neutral	33.7	500
62	Dunnigan clay	Mixed	Secondary alluvial	Semimature	Brown-gray	Saline	28.0	500
63	Esparto clay	Sedimentary	Secondary alluvial	Youthful	Light brown	Slightly acid	22.4	500
64	Farwell adobe clay	Basic igneous	Secondary alluvial	Youthful	Chocolate-brown	Neutral	25.3	500
65	Fresno light clay	Acid igneous	Secondary alluvial	Mature	Brown-gray	Alkaline	28.0	500
66	Imperial clay	Mixed	Secondary alluvial	Recent	Light gray	Alkali	28.8	500
67	Landlow adobe clay	Basic igneous	Secondary alluvial	Mature	Dark brown	Calcareous subsoil	22.1	500
68	Madera clay	Acid igneous	Secondary alluvial	Mature	Brown	Neutral	25.0	500
69	Merced adobe clay	Acid igneous	Secondary alluvial	Semimature	Black	Neutral	49.1	500
70	Montezuma adobe clay	Sedimentary	Secondary alluvial	Semimature	Gray-black	Neutral	28.0	500
71	Montezuma adobe clay	Sedimentary	Secondary alluvial	Semimature	Gray-black	Neutral	33.0	500
72	Panoche adobe clay	Sedimentary	Secondary alluvial	Recent	Brown-gray	Calcareous	25.0	500
73	Porterville adobe clay	Basic igneous	Secondary alluvial	Immature	Chocolate-brown	Neutral	26.5	500
74	Salinas clay	Mixed	Secondary alluvial	Immature	Dark brown-gray	Neutral	20.0	500
75	Sites adobe clay	Sedimentary	Primary	Semimature	Red-brown	Moderately acid	22.5	500
76	Stockton adobe clay	Basic igneous	Secondary alluvial	Mature	Gray-black	Basic	32.0	500
77	Tulare clay	Mixed	Secondary alluvial	Immature	Light gray	Calcareous	28.0	500
78	Willows adobe clay	Sedimentary	Secondary alluvial	Semimature	Dark brown	Neutral	30.5	500
79	Yolo adobe clay	Sedimentary	Secondary alluvial	Recent	Brown	Neutral	26.6	500
80	Yolo clay	Sedimentary	Secondary alluvial	Recent	Brown	Neutral	26.3	500

† Data given in this column were taken from (*14*, *15*, and *16*). They apply to the soil types in general. No such determinations were made on the samples collected.

California soils. The range of concentrations used in these early trials was not sufficient to show what changes in toxicity take place with repeated cropping; furthermore, two of the soils used were not quite typical. The Stockton adobe clay for the first experiment was taken near a drainage ditch and proved to be mostly subsoil that behaved anomalously. The Columbia fine sandy loam was not so fertile and was coarser-textured than that used in later tests. A retest was therefore devised to correct these difficulties.

When the results, which are presented in a later section, were compared with those of the previous experiment, it was impossible to formulate general relations between toxicity and soil type suitable for prescribing dosages. Therefore a simpler test was devised that could be used simultaneously on many soils. The results of these simple comparative tests form the main subject of this report.

MATERIALS AND METHODS

Selection and Sampling of Soils.—In conjunction with the Division of Soil Technology at Davis, sampling areas for type soils were located on soil-survey maps. The samples, taken from the top 4 inches after removal of the surface débris, were collected during the summer dry season, and wherever possible, near fence lines or from similar locations where they had not recently been disturbed.

After transportation to Davis, they were pulverized to pass a ¼-inch screen and were stored in burlap bags in a dry place until used. Table 1 presents descriptive data obtained from various sources (*14, 15, 16*). A casual survey will indicate the wide variety tested. Collected throughout the length and breadth of the state, the soils illustrate almost every textural grade, mode of formation, color, and reaction; and most important agricultural soils are represented by one or more types.

Biological Testing of Toxicity.—The biological testing method used in studying arsenic toxicity in these soils has been described (*6, 9*). It consists of growing a series of cultures in No. 2 cans in the greenhouse. The air-dry soils are weighed into the cans, which have been tared, bits of coarse gravel being added to bring them to a standard weight. The arsenic is added in solution in the water used to bring the soils to field capacity. Dry soil and solution are rapidly mixed, each in 3 successive portions to insure uniform distribution. After moistening, 13 Kanota oat seeds are planted in each can; and wrapping paper is laid over the cultures to prevent drying. The paper is removed as soon as the seeds germinate, and the plants are thinned to 10 at the end of the first week of growth. There-

after, they are watered as required by growth, sunshine, and humidity. After 30 days, they are cut off at the soil level. The fresh weights of the tops are recorded, and are used as a measure of toxicity of the arsenic applied.

The stock arsenic solution is prepared by mixing 4 parts of screened, dry, arsenic trioxide, 1 part of C.P. stick caustic soda, and 3 parts of water. When heated slightly, this mixture goes into solution, giving a clear sirupy liquid containing 50 per cent As_2O_3 by weight. The diluted solution for application to the soils is prepared by making up 10 grams of this to a liter. The resulting solution, containing 5,000 p.p.m. of As_2O_3, is measured out with a burette and further diluted to the appropriate strength. This concentration of 5,000 p.p.m. is particularly convenient in making up cultures in 500-gram lots of soil, since the number of cubic centimeters added, multiplied by 10, gives the p.p.m. based on the weight of the air-dry soil.

The concentration series used in the tests on the 80 soils ran as follows: 0, 15, 40, 80, 140, 220, 340, 490, 680, and 920 p.p.m. As_2O_3 in the air-dry soil. All series were run in triplicate. In determining the amount of water required to moisten these soils, a simple method has been used. When 50-gram lots of the soils have been weighed into test tubes, water is added—2.5 cc, 5.0 cc, 7.5 cc, or 10.0 cc, according to the textural grade of the soil. After 24 hours, the depth of the soil column moistened is measured, and the volume of water necessary to wet 100 grams of soil calculated. By an appropriate factor, the volume needed in the cultures is determined. This method has proved simpler and more satisfactory than determining the moisture equivalent, since it allows for the moisture present in the air-dry soil and for factors of soil preparation that must be considered in the latter method.

Data on the water-holding capacities of the soils and on the weights of soil used in the cultures are reported in table 1.

EXPERIMENTAL RESULTS

Retests on Four Soil Types.—In order to remedy some of the difficulties experienced in the initial trial, a more extended experiment was set up, with an expanding series of concentrations ending with cultures containing 3,000 p.p.m.

The soils for this retest were more carefully selected than those in the earlier experiment. The Stockton adobe clay was carefully selected from an area along a fence, undisturbed for many years and never affected except by shallow plowing. The Columbia fine sandy loam of this and

later experiments was somewhat more fertile and a bit finer-textured than that of the previous tests. The Yolo clay loam and the Fresno sandy loam were the same.

TABLE 2

TOXICITY OF SODIUM ARSENITE IN 4 CALIFORNIA SOILS AS SHOWN BY GROWTH OF INDICATOR PLANTS; EFFECTS OF TIME AND CROPPING*

Sodium arsenite expressed as p.p.m. As_2O_3 in air-dry soil	Yolo clay loam		Stockton adobe clay		Fresno sandy loam		Columbia fine sandy loam	
	Height	Fresh weight	Height	Fresh weight	Height	Fresh weight	Height	Fresh weight
First run, harvested December 29, 1933								
p.p.m.	*cm*	*gm*	*cm*	*gm*	*cm*	*gm*	*cm*	*gm*
10	36	8.5	18	1.9	29	5.1	32	6.8
30	35	8.0	16	1.7	26	3.6	32	6.7
60	35	7.8	14	1.2	20	1.9	31	5.9
100	35	7.5	12	1.0	8	0.7	30	5.5
150	33	6.6	10	0.9	7	0.5	27	4.8
210	33	6.2	9	0.8	5	0.1	20	2.6
280	31	4.9	10	0.8	0	0.0	9	0.7
360	26	3.3	8	0.6	0	0.0	7	0.5
450	16	2.0	7	0.5	0	0.0	3	0.3
550	8	0.8	5	0.4	0	0.0	0	0.0
660	8	0.6	4	0.3	0	0.0	0	0.0
780	6	0.4	5	0.3	0	0.0	0	0.0
910	4	0.2	4	0.2	0	0.0	0	0.0
1,050	3	0.1	4	0.1	0	0.0	0	0.0
Check	36	9.1	21	2.0	28	5.5	33	7.3
Third run, harvested May 31, 1934								
p.p.m.	*cm*	*gm*	*cm*	*gm*	*cm*	*gm*	*cm*	*gm*
10	34	9.6	21	3.4	26	4.4	29	5.4
30	34	9.8	21	3.2	26	4.3	28	6.1
60	34	9.8	22	3.0	25	3.9	28	5.6
100	32	8.4	20	2.3	23	3.4	26	4.5
150	30	6.2	19	2.1	20	2.7	21	2.7
210	28	5.3	15	1.4	16	1.8	18	2.0
280	24	3.5	11	1.0	13	1.5	14	1.5
360	21	2.5	9	0.5	14	1.3	12	1.2
450	18	2.1	8	0.4	9	0.8	11	0.9
550	16	1.8	8	0.3	7	0.5	10	0.6
660	13	1.3	8	0.4	6	0.2	9	0.5
780	11	0.9	8	0.4	6	0.3	8	0.3
910	11	0.9	8	0.4	5	0.1	6	0.2
1,050	11	0.6	8	0.4	0	0.0	6	0.1
1,200	10	0.5	7	0.2	0	0.0	0	0.0
1,360	9	0.5	7	0.1	0	0.0	0	0.0
1,530	8	0.2	0	0.0	0	0.0	0	0.0
1,710	7	0.1	0	0.0	0	0.0	0	0.0
Check	30	8.1	20	3.1	23	4.3	26	5.1

* Each value given is an average of 5 replicates.

TABLE 2—(*Concluded*)

Sodium arsenite expressed as p.p.m. As_2O_3 in air-dry soil	Yolo clay loam		Stockton adobe clay		Fresno sandy loam		Columbia fine sandy loam	
	Height	Fresh weight	Height	Fresh weight	Height	Fresh weight	Height	Fresh weight
Fifth run, harvested January 9, 1935								
p.p.m.	*cm*	*gm*	*cm*	*gm*	*cm*	*gm*	*cm*	*gm*
10	29	5.9	21	2.4	25	3.7	28	4.8
30	30	6.3	22	2.8	26	3.5	27	4.8
60	29	6.0	22	2.6	25	3.5	28	5.3
100	29	6.5	23	3.4	26	3.5	29	5.4
150	31	6.9	25	3.7	26	3.4	29	5.4
210	31	6.4	23	3.0	23	2.8	27	4.6
280	30	6.1	22	2.8	23	2.6	25	3.4
360	27	4.9	17	1.9	19	1.9	23	2.7
450	26	4.5	18	1.7	15	1.4	20	1.9
550	24	3.7	12	1.2	11	0.8	18	1.5
660	23	3.1	13	1.3	11	0.7	17	1.4
780	22	2.7	12	1.2	10	0.6	14	1.1
910	21	2.4	11	1.2	9	0.5	13	0.9
1,050	19	2.0	11	1.1	9	0.5	11	0.7
1,200	17	1.5	11	1.0	8	0.3	11	0.6
1,350	15	1.2	11	0.8	8	0.3	10	0.5
1,530	13	1.0	10	0.7	7	0.2	9	0.4
1,710	12	0.9	10	0.5	6	0.1	8	0.4
1,900	12	0.9	9	0.5	6	0.1	8	0.3
2,100	11	0.7	8	0.5	5	0.1	7	0.3
2,310	10	0.7	8	0.4	0	0.0	7	0.3
2,530	9	0.5	7	0.3	0	0.0	7	0.3
2,760	9	0.5	7	0.3	0	0.0	6	0.3
3,000	9	0.5	6	0.3	0	0.0	6	0.3
Check	26	5.0	20	3.0	25	3.5	26	4.5
Seventh run, harvested November 14, 1935								
p.p.m.	*cm*	*gm*	*cm*	*gm*	*cm*	*gm*	*cm*	*gm*
150					16	1.9		
210					15	1.9	18	2.7
280			14	1.8	14	1.6	16	1.8
360	24	5.4	14	1.6	12	1.5	14	1.5
450	24	4.8	12	1.2	11	1.1	13	1.3
550	23	3.9	10	1.0	9	0.7	12	1.0
660	21	3.1	10	1.0	8	0.6	10	0.9
780	20	2.7	10	1.1	8	0.5	9	0.7
910	18	2.3	11	1.1	7	0.4	9	0.6
1,050	15	1.7	11	1.2	7	0.4	7	0.4
1,200	14	1.5	11	1.1	6	0.3	5	0.2
1,360	13	1.4	10	1.0	6	0.3	4	0.1
1,530	12	1.1	8	0.8	5	0.2	4	0.1
1,710	10	1.1	7	0.6	5	0.2	4	0.1
1,900	10	0.8	7	0.4	4	0.2	3	0.1
2,100	8	0.6	6	0.4	3	0.1	3	0.1
2,310	7	0.5	5	0.2	0	0.0	0	0.0
2,530	7	0.4	5	0.2	0	0.0	0	0.0
2,760	6	0.5	4	0.2	0	0.0	0	0.0
3,000	6	0.4	3	0.1	0	0.0	0	0.0
Check	21	4.2	15	2.2	16	2.3	18	3.0

When complete, this experiment contained 24 concentrations and 4 checks, each consisting of 5 replicates. Similar series were established at about the same time for sodium chlorate and borax. The first 3 crops on the chlorate series were reported earlier (*6*), as were the first, third, and fifth crops of the borax tests (*9*). The first, third, fifth, and seventh crops of the present experiment on the arsenic series are given in table 2. In each run only the cultures having growth in one or more of the soils are reported, all higher concentrations having no growth. By the fifth run all concentrations in the Yolo and Stockton soils had so greatly decreased in toxicity that plants survived in them. Since the lower concentrations were producing crops as heavy as the checks or heavier, the first 4 were not included in the seventh run in table 2; and even higher concentrations were omitted in 3 of the soils.

The most noticeable result of the retest is the difference in behavior of the Stockton soil. Though producing a low yield, the plants survived through the lowest 13 concentrations; a fact indicating a toxicity similar to that of the Yolo clay loam. The change in toxicity, furthermore, practically kept pace with that of the Yolo soil. Evidently the results reported earlier (*6*) gave an inaccurate picture of the toxicity in adobe soils.

Tests on Eighty Soils.—Yield data on the eighty soils tested are presented in table 3. Obviously the toxicity results follow a definite pattern, toxicity being highest in the sands and lowest in the clays. There are a few notable exceptions, later to be considered in detail. The general relation may be more easily scrutinized in the summary in table 4, where averages for the 5 soil groups are compiled.

The water-holding capacities of the various soil groups, as shown in these averaged results, may be correlated with textural grade; and the arsenic toxicities show a related change. Conceivably, certain factors that enable the soil to hold water against the force of gravity are involved in the availability of applied arsenic to plants.

For comparing soil groups, a series of toxicity values have been calculated, based upon the yield of the untreated checks; these results, presented in table 4, are graphed in figure 1. Although the numbers in these averages are not great enough to give perfectly smooth curves and although the exceptional results on a few individual soils tend in places to overshadow the general relations, the correlation of toxicity and textural grade is obvious. The expression of this relation, regardless of the crops produced, is the principal finding in this study.

The relation of toxicity to textural grade is further illustrated by the crops in Oakley sand, Farwell loam, and Aiken clay loam shown in figure 2. These series all contain a 5 p.p.m. culture; and all concentrations being

TABLE 3

Toxicity of Sodium Arsenite in 80 California Soils as Shown by Growth of Indicator Plants

No.	Soil type	Date of harvest	Arsenic concentration—As_2O_3 in p.p.m. basis air-dry soil									
			0	15	40	80	140	220	340	490	680	920
			Fresh weight of plants									
	Sands											
			gm	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*
1	Holland loamy gravelly sand	Dec. 23, 1934	1.8	2.0	1.5	1.3	0.6	0.3	*			
2	Niland gravelly sand	June 5, 1935	1.0	0.7	0.4			*				
3	Oakley sand	Jan. 10, 1936	1.9	1.6	1.3	0.8	0.4	*				
4	Rositas fine sand	June 5, 1935	1.2	1.1	0.9	0.1		*				
5	Superstition gravelly sand	June 5, 1935	1.6	1.3	1.4	0.9	0.1	*				
6	Tujunga sand	June 4, 1935	0.5	0.4	0.6	0.1	*					
	Gravelly and sandy loams											
			gm	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*
7	Aiken gravelly loam	Dec. 23, 1934	1.6	1.7	1.6	1.8	1.7	1.8	1.6	0.4	0.3	0.1
8	Arbuckle gravelly sandy loam	Dec. 23, 1934	2.1	2.3	1.8	1.9	0.9	0.3	*			
9	Chualar fine sandy loam	Jan. 10, 1936	5.3	4.9	2.8	1.9	0.1		*			
10	Columbia fine sandy loam	June 4, 1935	4.4	4.8	4.0	3.0	1.5	0.3	*			
11	Corning gravelly loam	Dec. 23, 1934	1.6	1.5	1.4	1.2	0.8	0.3	0.2	*		
12	Delano fine sandy loam	Jan. 10, 1936	4.5	4.5	3.9	2.6	0.9	0.7	0.1			
13	Foster fine sandy loam	Jan. 10, 1936	3.3	2.8	0.4	0.1		*				
14	Fresno sandy loam	June 4, 1935	3.0	2.2	1.4	0.1	*					
15	Greenfield coarse sandy loam	Jan. 10, 1936	4.3	3.8	1.2	0.3	0.1	*				
16	Hanford sandy loam	Jan. 10, 1936	4.3	4.0	2.5	0.7	0.2	0.1	*			
17	Hanford fine sandy loam	Jan. 10, 1936	4.0	3.4	2.9	1.0	0.2	*				
18	Meloland fine sandy loam	June 5, 1935	1.9	1.9	1.5	1.0	0.2	*				
19	Merced fine sandy loam	Jan. 10, 1936	4.4	3.8	3.1	1.7	0.4	*				
20	Oakdale coarse sandy loam	Jan. 10, 1936	3.0	2.8	2.4	0.8	0.1		*			
21	Ramona sandy loam	June 5, 1935	3.0	3.0	2.6	1.6	0.3			*		
22	Redding gravelly loam	Jan. 10, 1936	3.3	3.1	2.8	2.5	2.1	0.7	0.1	*		
23	Rocklin sandy loam	Dec. 23, 1934	1.5	1.3	1.2	0.8	0.5	0.2	0.1		*	
24	Salinas fine sandy loam	Jan. 10, 1936	3.7	3.5	3.0	1.9	1.3	0.5	*			
25	Sierra gravelly loam	Dec. 23, 1934	1.8	1.9	1.6	1.8	1.6	1.5	0.7	0.6	0.2	*
26	Sierra sandy loam	Dec. 23, 1934	3.2	3.1	2.7	2.4	1.2	0.3	0.1	*		
27	Sites fine sandy loam	Dec. 23, 1934	1.9	1.9	1.4	1.3	0.7	0.2	0.1	*		
28	Tulare fine sandy loam	Jan. 10, 1936	2.1	1.8	1.3	0.3	0.2		*			
29	Yolo fine sandy loam	Dec. 22, 1934	3.5	3.3	3.1	2.9	2.6	0.6	0.3	0.2	*	
	Loams											
			gm	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*
30	Egbert loam	June 4, 1935	3.9	3.4	3.2	2.7	2.2	1.6	1.0	0.2	*	
31	Farwell loam	Jan. 10, 1936	7.3	7.2	6.1	5.4	3.2	1.0	0.1	*		
32	Gridley loam	Jan. 10, 1936	2.5	2.7	2.6	2.5	2.5	0.5	0.2			*
33	Honcut loam	Jan. 10, 1936	4.1	3.8	3.4	2.5	1.3	0.3	0.1	*		
34	Madera loam	June 5, 1935	3.2	2.7	2.6	2.2	1.7	0.5	0.3		*	
35	Panoche light loam	Jan. 10, 1936	2.7	2.4	2.2	0.8	0.2	0.2		*		
36	Pinole loam	Jan. 10, 1936	1.4	1.4	1.4	1.2	0.9	0.7	0.5		*	

* Seeds in cultures at this and higher concentrations failed to germinate. Fresh weight of plants in cultures between reported weight and point of no germination was less than 0.1 gram.

TABLE 3—(*Concluded*)

No.	Soil type	Date of harvest	Arsenic concentration—As_2O_3 in p.p.m. basis air-dry soil									
			0	15	40	80	140	220	340	490	680	920
			Fresh weight of plants									
	Loams—(*Continued*)											
			gm	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*
37	Placentia light loam.	June 4, 1935	2.7	2.4	2.2	1.7	0.1		*			
38	Pleasanton loam . ..	Jan. 10, 1936	4.4	4.2	4.0	2.4	0.6	0.2	*			
39	Pond heavy loam. .	Jan. 10, 1936	0.0		*							
40	San Joaquin loam ..	June 5, 1935	3.4	2.6	2.7	1.8	1.3	0.6	0.2		*	
41	Tehama loam......	Jan 10, 1936	2.6	2.4	2.2	0.8	0.2		*			
42	Vina loam..	Jan. 10, 1936	3.3	3.6	3.4	3.2	3.0	0.9	0.2		*	
43	Yolo loam. ..	Dec. 22, 1934	3.8	3.6	3.5	3.1	2.2	0.3	0.2	0.1	*	
	Silt and clay loams											
			gm	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*
44	Aiken clay loam .	Jan. 10, 1936	1.0	1.0	0.9	0.9	0.9	0.8	0.7	0.6	0.6	0.3
45	Antioch clay loam. . .	Jan. 10, 1936	3.9	4.5	3.9	4.0	2.8	1.2	0.5	*		
46	Arbuckle clay loam . .	Dec. 23, 1934	2.4	2.4	1.9	1.9	1.1	1.3	0.3	0.1	*	
47	Chino silty clay loam	June 5, 1935	1.6	1.5	1.5	1.4	0.7	0.1		*		
48	Columbia silty clay loam.... ..	June 5, 1935	4.2	3.8	3.7	2.8	2.7	2.4	2.4	1.5	1.1	0.3
49	Mariposa silt loam ..	Dec. 22, 1934	2.0	1.9	1.8	1.4	1.5	0.7	0.6	0.3	*	
50	Marvin silty clay loam .	Jan. 10, 1936	3.4	3.9	3.5	3.1	1.9	0.7	0.4		*	
51	Ramada silt loam	Jan. 10, 1936	6.5	6.3	6.2	3.8	1.6	0.3	0.1	*		
52	Sacramento clay loam	June 5, 1935	4.1	4.2	3.9	2.7	2.0	0.8	0.5			*
53	Yolo silt loam .	Dec. 22, 1934	8.9	8.6	6.9	6.5	3.4	0.9	0.3	0.2	*	
54a	Yolo clay loam	Dec. 22, 1934	9.8	9.4	9.5	8.8	5.1	1.4	0.4	0.2	*	
54b	Yolo clay loam .	June 4, 1935	8.7	8.8	7.6	6.9	5.8	4.0	0.6	0.4	*	
	Clays											
			gm	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*
55	Alamo adobe clay .	Jan. 10, 1936	1.7	1.7	1.6	1.4	1.3	0.7	0.5	0.3	0.2	0.1
56	Anita adobe clay	Jan. 10, 1936	2.1	2.1	1.8	1.7	1.2	1.1	0.9	0.7	0.2	0.1
57	Capay adobe clay	Dec. 23, 1934	3.7	3.7	3.4	3.5	3.1	2.1	1.4	1.5	0.5	0.5
58	Clear Lake adobe clay .	June 4, 1935	1.6	1.5	1.4	1.1	1.1	0.7	0.4	0.2	0.1	
59	Conejo adobe clay	Jan. 10, 1936	1.8	1.9	1.5	1.2	0.8	0.8	0.7	0.5	0.3	0.2
60	Diablo adobe clay	Jan. 10, 1936	2.6	2.4	1.9	1.5	0.8	0.4	0.2	0.2	0.1	
61	Dublin adobe clay	Jan. 10, 1936	4.7	4.9	4.6	4.4	4.3	3.7	3.2	2.0	0.5	0.3
62	Dunnigan clay	Dec. 23, 1934	1.8	1.8	1.6	1.1	0.3	0.3	0.2	0.2	*	
63	Esparto clay. .	Dec 22, 1934	4.4	4.5	4.0	3.9	3.2	2.5	1.4	0.5	0.1	
64	Farwell adobe clay	Jan. 10, 1936	2.5	2.4	2.4	1.9	1.6	1.2	0.8	0.7	0.3	0.1
65	Fresno light clay .	Jan. 10, 1936	1.0	1.5	1.3	0.6	0.4	0.3	0.2	0.2		
66	Imperial clay .	June 5, 1935	3.0	2.9	2.8	2.4	2.1	1.6	0.8	0.5		
67	Landlow adobe clay	Jan. 10, 1936	1.8	2.2	2.1	2.0	1.7	1.6	0.8	0.6	0.2	
68	Madera clay... .	Jan. 10, 1936	2.3	2.1	1.7	1.6	1.1	0.9	0.5	0.5	0.1	
69	Merced adobe clay.	Jan. 10, 1936	1.5	1.5	1.5	1.3	1.1	1.1	0.7	0.4	0.3	0.2
70	Montezuma adobe clay	June 4, 1935	2.3	2.1	1.8	1.2	1.1	0.9	0.5	0.3	0.2	
71	Montezuma adobe clay	Jan. 10, 1936	2.1	2.3	2.0	2.1	1.9	1.0	0.5	0.1	*	
72	Panoche adobe clay .	Jan. 10, 1936	8.1	8.1	6.9	6.6	5.4	3.7	2.5	0.7	0.4	0.2
73	Porterville adobe clay	Jan. 10, 1936	4.2	3.8	3.5	2.0	0.9	0.3	0.2	0.2	*	
74	Salinas clay	Jan. 10, 1936	3.5	3.5	3.3	3.2	1.1	0.3	0.2		*	
75	Sites adobe clay . .	Dec. 22, 1934	2.8	3.0	2.5	2.0	1.6	1.1	0.5	0.3	0.1	0.1
76	Stockton adobe clay .	April 24, 1936	2.9	2.3	1.8	1.8	1.5	0.8	0.6	0.4	0.1	
77	Tulare clay . .	Jan. 10, 1936	1.6	1.5	1.4	0.5	0.3	0.1	*			
78	Willows adobe clay .	Dec. 23, 1934	2.0	2.2	1.9	1.6	1.2	0.7	0.7	0.4	0.1	0.1
79	Yolo adobe clay.	Jan. 10, 1936	1.9	1.9	1.7	1.5	0.7	0.2	0.1		*	
80	Yolo clay....	Dec. 22, 1934	4.4	4.8	4.4	4.5	4.1	3.9	2.6	2.2	1.0	0.1

* Seeds in cultures at this and higher concentrations failed to germinate Fresh weight of plants in cultures between reported weight and point of no germination was less than 0.1 gram.

comparable in the 3 series, the heavier soils show obviously lower toxicities, while crop-producing power (table 3) varies in no regular way with textural grade.

Figure 3, showing 3 toxicity series in adobe soils, further illustrates this point. Although the crop yields vary widely, being high in the Panoche soil, intermediate in Dublin, and low in Merced (table 3), toxicities are strictly comparable in the 3 soils. These series lack the 5-p.p.m. cultures but have the 920-p.p.m. ones.

TABLE 4

SUMMARY OF RESULTS: TOXICITY OF SODIUM ARSENITE IN CALIFORNIA SOILS AS SHOWN BY GROWTH OF INDICATOR PLANTS

Soils	Water	Arsenic concentration—As_2O_3 in p.p.m. on the basis of air-dry soil									
		0	15	40	80	140	220	340	490	680	920
					Fresh weight of plants						
	per cent	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*
Sands	11 4	1 33	1 18	1 02	0 53	0 18	0 05				
Gravelly and sandy loams	14 3	3 12	2 88	2 20	1 46	0 77	0 33	0 14	0 05		
Loams	17 9	3 49	3 26	3 04	2 33	1 48	0 52	0 21	0 02		
Silt and clay loams	22 7	4 71	4 69	4 28	3 68	2 46	1 22	0 57	0.28	0 14	0 05
Clays	27 6	2 82	2 79	2 50	2 18	1 70	1 23	0 81	0 52	0 18	0 08
					Results expressed as a percentage of checks						
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Sands	11 4	100	88 8	76.7	30 6	13 5	3 8				
Gravelly and sandy loams	14 3	100	91 6	70 5	46 8	24 6	10 6	4 5	1 6		
Loams	17 9	100	93 5	87 1	66 8	42 4	14 9	6 0	0 6		
Silt and clay loams	22 7	100	99 5	91 0	78 1	52 3	25 9	12 1	5 9	3 0	1 1
Clays	27 6	100	99 2	88 6	77 4	60 3	43 6	28 7	18 4	6 4	2 8

A more detailed study of the data in table 3 shows many minor variations in toxicity within the groups designated on the basis of soil texture. Though the general relation shown between texture and toxicity is valuable, its usefulness would be enhanced if the exceptions could be explained and anticipated in the field, as is possible in several cases.

The soils most obviously out of agreement are Aiken gravelly loam, Aiken clay loam, and Columbia silty clay loam. The two Aiken soils—residual soils from basic igneous rock—are deep red. They have demonstrated an immense capacity to render phosphate unavailable and by analogy should do the same with arsenic. Tests in the field and greenhouse show this to be true. Earlier, the red iron oxide content of these soils was thought to explain their immense capacity to reduce arsenic tox-

icity (*10*). Judging from recent experiments, however, peculiar colloids at least partly account for this phenomenon. This property of rendering arsenic unavailable, though common to all red soils, is less pronounced in those from acid igneous rocks. Among the gravelly and sandy loams (in addition to the Aiken), the Corning, Delano, Redding, Rocklin, Sierra, and Sites soils all contain more or less of this material and all show relatively low toxicities. Incidentally, red iron oxide has been used to lower

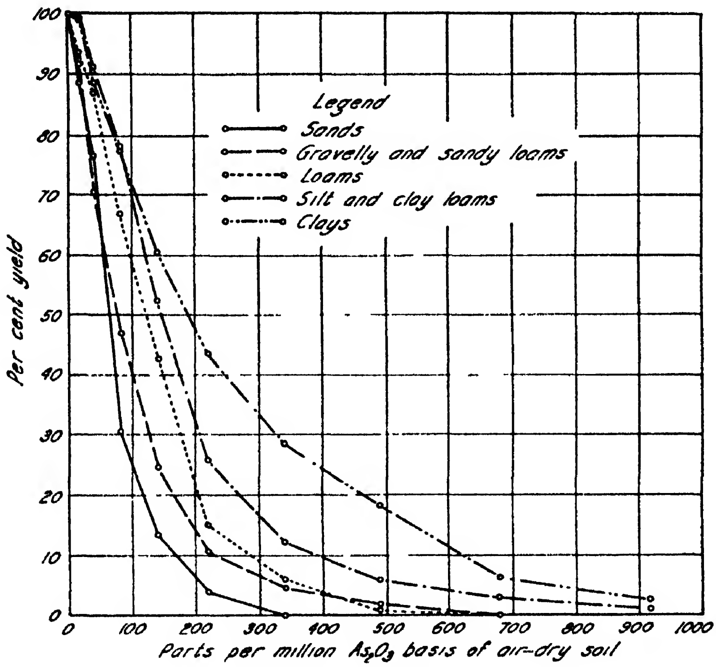

Fig. 1.—The relation between textural grade and arsenic toxicity from the summarized results of table 4.

arsenic solubility in filter beds made of soil (*10*). A great capacity for reducing arsenic toxicity is apparently characteristic of all red soils used in these tests, so that dosages must be set with this factor in mind, more arsenic being required than would ordinarily be applied on the basis of textural grade alone.

The Columbia soils, recent alluvial deposits from the floodwaters of the Sacramento River, come from a mixture of acid and basic igneous rocks from the Sierras with sedimentary rocks from the Coast Range. During deposition, the heavier particles settle out along the river banks, while the finer ones are deposited farther from the main channel. To obtain a silty clay loam it was necessary to visit the very edge of the alluvial deposits. The source of soil used in these experiments was a spot

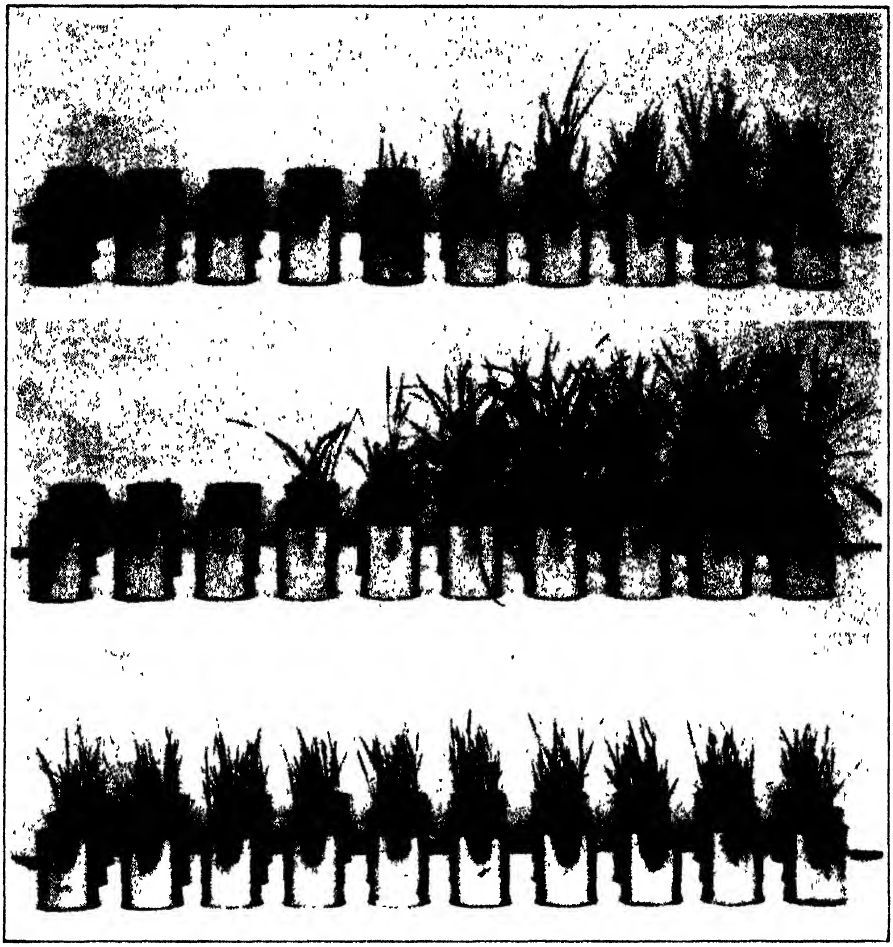

Fig. 2.—Culture series in Oakley sand, Farwell loam, and Aiken clay loam illustrating the relation between textural grade and arsenic toxicity. The As_2O_3 concentrations are 680, 490, 340, 220, 140, 80, 40, 15, 5, and 0 p.p.m., based on the air-dry soil.

about 3 miles west of Sacramento near the main highway, where the Columbia soil occurs as a thin layer about 18 inches deep, overlying an extremely heavy, black Sacramento clay. Such a soil might be expected to contain a large proportion of the colloidal fractions characteristic of the soils from the three available rock sources. Though the sample used contained considerable silt, it undoubtedly had, in addition, enough colloids from the red Aiken and Sierra soils and the brown Yolo soils to explain the low toxicity. The coarser Columbia fine sandy loam obtained only a few miles farther north consisted largely of fine sand and silt, without enough of these active colloids to give it an unusual behavior.

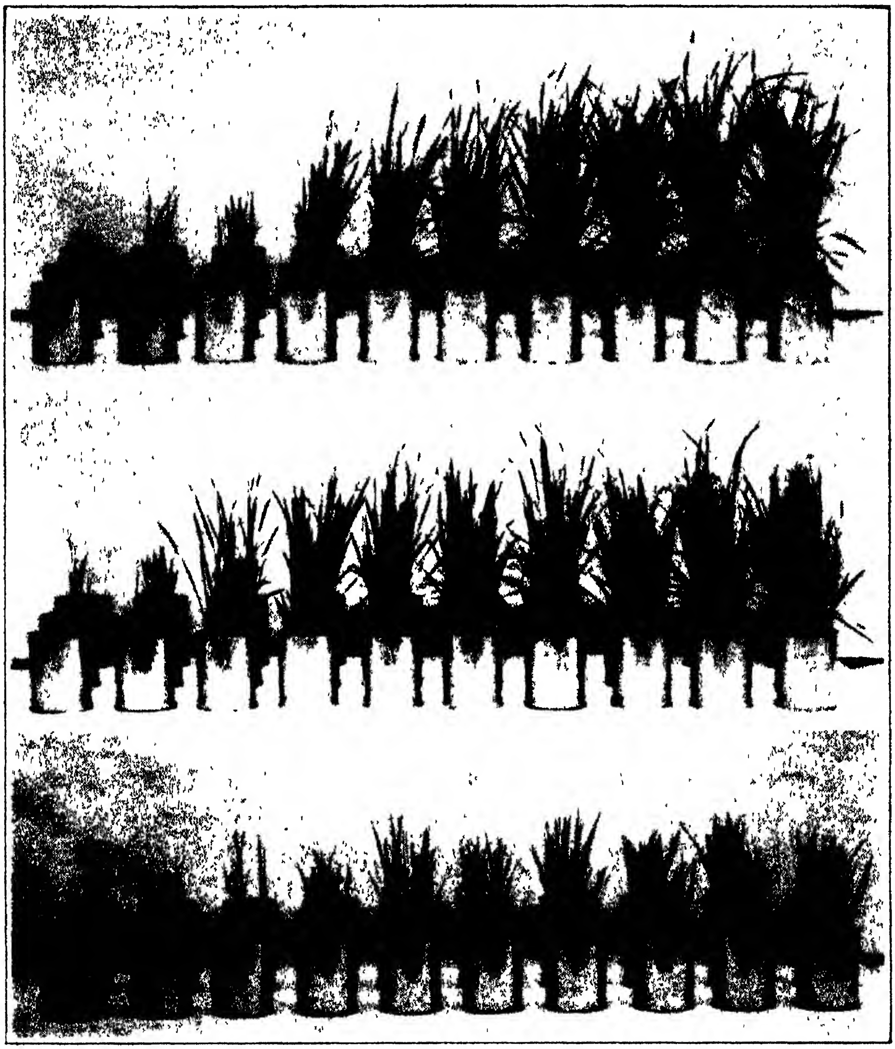

Fig. 3.—Culture series showing arsenic toxicity in Panoche adobe clay, Dublin adobe clay, and Merced adobe clay. Although fresh-weight yields of crops on these soils vary widely, the toxicity is approximately the same in all three. These series lack the 5 p.p.m. cultures. The As_2O_3 concentrations are 920, 680, 490, 340, 220, 140, 80, 40, 15, and 0 p.p.m.

Exceptional results are also shown by the Yolo soil in these experiments. Being easily obtainable, a full series of textural grades of this soil was used to show the effect of particle size within a single soil series. The choice of the Yolo series for this purpose was unfortunate in that the two lighter types act like heavy soils, whereas the adobe behaved like a lighter type. The behavior of the lighter types may be explained by their colloids, which have an extreme capacity to render arsenic unavailable.

This observation has been made for phosphates but has no important bearing on crop production, since phosphorus is apparently not deficient in Yolo soils. In soil sterilization with arsenic, however, this capacity is a critical factor, for Yolo soils in the field require heavy arsenic applications (*5*), and toxicity decreases rapidly after the initial treatment (*5, 6,* and table 2).

The abnormal behavior of the Yolo adobe clay is less readily explained. This soil required no more water for moistening than the clay and less than the clay loam. Though its colloids are of such a form as to give this soil adobe characteristics, the total colloid content may be less than that of the other two grades. As is shown in another paper in this issue (*13*, table 3), the capacity of this soil to render arsenic insoluble after several weeks is less than that of the clay.

DISCUSSION

To be widely applicable, the general relations brought out by these studies should rest upon chemical interpretation and practical confirmation through field-plot testing. Chemical studies on a number of these soils appear in an accompanying paper (*13*); a few field-plot tests will be mentioned here, but plot results will be presented more completely in a later paper.

The general problem of toxicity measurement has been discussed in detail by Cook (*2, 3, 4*). The present method of measuring and reporting height and fresh weight of the indicator plants 30 days after planting in soils moistened with the herbicide solutions was standardized several years ago and has proved entirely satisfactory. These values, however, are only comparative; for practical use they must be calibrated by checking against graded series of treated plots. Although this checking has not been extensively done, growing of the indicator plants on soils from plots having known degrees of sterility has shown that yields of 0.2 gram or less per can represent practical sterilization. Such comparisons seem sufficiently reliable to justify a tentative schedule of dosages, offered later in this paper.

Since fresh weight is by far the more valuable of the two toxicity criteria used, height has been omitted from tables 3, 4, and 5. Obviously, relative growth rate (*4*) cannot be used as a criterion of toxicity in the type of testing reported here, since the soil cultures could not be returned to the original weight for determination of growth increments The work involved in repeated weighings on the large numbers of cultures run simultaneously in these tests was, furthermore, not feasible.

Before making the more practical interpretations, the errors and limitations of the biological testing methods used should be considered. Since the greenhouse in which these tests were conducted had only partial temperature control and practically no control of light, humidity, and length of day, results from culture series run at different seasons vary. Table 5 reports tests conducted at different dates upon the same soils. These data illustrate variations in toxicity resulting from the lack of constant culture conditions and, in the cases of Yolo clay loam and Stockton adobe clay, from the use of different samples. They emphasize the desirability of conducting comparative tests simultaneously on as many samples as possible. The soils in table 3 were tested in 3 lots, 2 of 20 each and 1 of 40. Though testing all 80 at one time would have been better, the work presents practical difficulties. Had it been done, the general relation shown between texture and toxicity would probably not have been appreciably changed.

The moisture conditions of the cultures are another matter for consideration. One might think that allowing the cultures to dry down periodically to the permanent wilting point would increase the arsenic concentration and hence the toxicity. In certain series, the moisture was varied in a number of soils (footnotes ¶ and ||, table 5). Judging from the results, arsenic toxicity is not seriously affected by the method of watering. This matter will be discussed more fully in a companion paper (*13*).

The biological test is definitely limited in scope by the sensitivity of the indicator plant. Since, however, the practical application of the results is in weed control, this drawback is not serious. The biological method, furthermore, gives a direct index to the availability and hence to the toxicity or crop-limiting power of the toxicant, which is impossible to obtain by chemical analysis. Considering the easy operation and the simple, inexpensive equipment needed, this method is very practical for testing toxicity of herbicides in soils.

RECOMMENDED DOSAGES OF ARSENIC FOR SOIL STERILIZATION

Clearly, these studies show that arsenic dosages for soil sterilization will vary between wide limits. Recommendations can at best be only approximate because of the complex relations between toxicity as related to availability, permanence as affected by leaching, and susceptibility as determined by the arsenic tolerance of the weed species concerned. Table 6 presents a dosage schedule based on plots and the present toxicity

TABLE 5

COMPARATIVE RESULTS OF TOXICITY TESTS ON REPEATED RUNS WITH SODIUM ARSENITE IN CALIFORNIA SOILS

Soil type	Run No.	Date of harvest	Arsenic concentration—As_2O_3 in p.p.m. on the basis of air-dry soil									
			0	15	40	80	140	220	340	490	680	920
			Fresh weight of plants									
			gm	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*
Columbia fine sandy loam	1*	June 10, 1933*	4.6	4.6	4.4	4.1	3.0	1.7	0.3	0.1		
	2†	Dec. 29, 1933†	7.3	6.8	6.5	5.7	5.0	2.2	0.5	0.1		
	3‡	June 4, 1935‡	4.4	4.8	4.0	3.0	1.5	0.3				
	4§	Apr. 24, 1936§	13.5	11.1	9.9	5.5	1.4	0.2				
Delano fine sandy loam	1‡	Jan. 10, 1936‡	4.5	4.5	3.9	2.6	0.9	0.7	0.1			
	2¶	Mar. 16, 1937¶	2.9	2.5	1.8	0.7	0.3	0.5				
	3‖	Mar. 16, 1937‖	2.6	2.2	1.7	0.6	0.3	0.2				
Fresno sandy loam	1*	June 10, 1933*	2.9	2.4	1.4	0.6	0.4	0.2				
	2†	Dec. 29, 1933†	5.5	4.6	3.1	1.2	0.5	0.1				
	3‡	June 4, 1935‡	3.0	2.2	1.4	0.1						
	4§	Apr. 24, 1936§	7.2	4.0	2.6	0.5	0.2					
	5¶	Mar. 16, 1937¶	4.7	3.8	2.2	0.7	0.2	0.2				
	6‖	Mar. 16, 1937‖	4.4	4.3	2.3	0.6	0.2					
Greenfield coarse sandy loam	1‡	Jan. 10, 1936‡	4.3	3.8	1.2	0.3	0.1					
	2¶	Mar. 16, 1937¶	3.2	2.7	1.1	0.7	0.3					
	3‖	Mar. 16, 1937‖	1.4	1.7	0.9	0.6	0.2					
Sierra sandy loam	1‡	Dec. 23, 1934‡	3.2	3.1	2.7	2.4	1.2	0.3	0.1			
	2¶	Mar. 16, 1937¶	3.0	2.5	2.3	1.6	0.7					
	3‖	Mar. 16, 1937‖	2.3	2.2	2.0	0.3	0.1					
Yolo fine sandy loam	1‡	Dec. 22, 1934‡	3.5	3.3	3.1	2.9	2.6	0.6	0.3	0.2		
	2§	Apr. 24, 1936§	5.4	5.5	4.7	4.4	2.4	0.4				
Egbert loam	1‡	June 4, 1935‡	3.9	3.4	3.2	2.7	2.2	1.6	1.0	0.2		
	2¶	Mar. 16, 1937¶	8.2	8.6	8.4	8.5	5.7	3.7	2.3	1.1	1.1	0.4
	3‖	Mar. 16, 1937‖	5.8	5.2	5.6	5.3	4.1	3.8	1.6	0.6	0.7	0.1
Aiken clay loam	1‡	Jan. 10, 1936‡	1.0	1.0	0.9	0.9	0.9	0.8	0.7	0.6	0.6	0.3
	2§	Apr. 24, 1936§	3.2	3.5	3.4	2.9	2.4	2.2	2.1	1.8	1.1	0.7
Arbuckle clay loam	1‡	Dec. 23, 1934‡	2.4	2.4	1.9	1.9	1.1	1.3	0.3	0.1		
	2§	Apr. 24 1936§	4.5	4.2	3.6	3.3	3.0	2.9	1.5	0.4		
Sacramento clay loam	1‡	June 5, 1935‡	4.1	4.2	3.9	2.7	2.0	0.8	0.5			
	2¶	Mar. 16, 1937¶	5.2	5.3	5.4	4.9	4.0	3.7	2.6	1.9	0.8	0.1
	3‖	Mar. 16, 1937‖	2.1	3.5	3.5	3.1	2.6	2.8	2.1	0.7	0.1	

* By interpolation from Crafts (*6*).
† By interpolation from table 2.
‡ From table 3.
§ From Crafts (*7*).
¶ From Rosenfels and Crafts (*13*); watered daily.
‖ From Rosenfels and Crafts (*13*); watered as needed

TABLE 5—(*Concluded*)

Soil type	Run No.	Date of harvest	Arsenic concentration—As_2O_3 in p.p.m. on the basis of air-dry soil									
			0	15	40	80	140	220	340	490	680	920
			Fresh weight of plants									
			gm	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*
Yolo clay loam	1*,[a]	June 10, 1933*,[a]	8.6	8.6	7.6	7.2	5 7	4.8	2.3	1.0	0 3	
	2†,[a]	Dec. 29, 1933†,[a]	9.1	8.4	7 9	7.6	6.7	5.8	3.7	1 5	0.5	0.2
	3‡,[b]	Dec. 22, 1934‡,[b]	9.8	9.4	9 5	8 8	5 1	1.4	0 4	0.2		
	4‡,[b]	June 4, 1935‡,[b]	8.7	8 8	7.6	6.9	5 8	4 0	0 6	0 4		
	5§,[c]	Apr 24, 1936§,[c]	18 6	17.5	14 2	12.4	8.9	5.4	0 3			
Stockton adobe clay	1*,[a]	June 10, 1933*,[a]	2 9	2.3	2.0	1 6	0.5	0 2	0.1	0 1	0 1	
	2†,[b]	Dec. 29, 1933†,[b]	2 0	1.8	1 5	1.1	0 9	0 8	0 7	0 5	0 3	0.2
	3‡,[c]	Apr. 24, 1936‡,[c]	2.9	2 3	1 8	1.8	1 5	0 8	0 6	0 4	0 1	
Yolo clay....	1‡	Dec. 22, 1934‡	4 4	4.8	4 4	4 5	4 1	3 9	2.6	2.2	1.0	0.1
	2¶	Mar. 16, 1937¶	4 1	3.8	3 6	3.1	2 8	2 5	0 9	1 0	0.1	
	3‖	Mar. 16, 1937‖	3 5	3.3	3.2	3.3	2 9	1 5	1 3	1.3	0 5	0.3
Yolo adobe clay......	1‡	Jan. 10, 1936‡	1.9	1 9	1 7	1 5	0.7	0.2	0.1			
	2¶	Mar. 16, 1937¶	3 2	3 3	3 3	2 8	1 6	1 1	0.1	0 1		
	3‖	Mar. 16, 1937‖	2 8	2 8	2 7	2 8	2 0	1 7	0 1			

* By interpolation from Crafts (*6*).

† By interpolation from table 2.

‡ From table 3.

§ From Crafts (*7*).

¶ From Rosenfels and Crafts (*13*); watered daily.

‖ From Rosenfels and Crafts (*13*); watered as needed.

[a] Soil sample collection in 1932

[b] Soil sample collection in 1933.

[c] Soil sample collection in 1935.

studies in the greenhouse. The groupings are somewhat arbitrary and require liberal interpretation to meet specific problems.

In the high-toxicity group are the coarse, gritty soils having little colloidal matter. Such soils not only are common on the alluvial fans and upper flood-plain areas of the large valleys of California but also occur as surface material in many foothill and mountain regions, on old valley fills, bench lands, wind-modified areas, and heavily leached areas. Lands of this type are developed by man by the deposition of gravel and rock in roadways, railroad roadbeds, parking areas, and various yards and lots used for stacking lumber and the raw materials for manufacture. Vast areas of such lands could be profitably sterilized with arsenic with little poisoning hazard to livestock and at a great saving of hand labor.

In the intermediate-toxicity range lie the loams, silt loams, and those clay loams that are developed directly from acid igneous rocks or highly weathered from other rocks; also lighter soils from basic igneous and sedimentary rocks. Many agricultural soils of California lie in this range,

and their successful sterilization requires appreciably heavier dosages than with the high-toxicity type.

The heavier soils of the clay and adobe clay types, together with the intermediate red soils from basic igneous rocks and brown soils from sedimentary rocks, demand heavy arsenic dosages; and their successful sterilization requires special technique in application.

In soil sterilization, two factors should be kept in mind: first, the immediate effect of the application; second, the persistence of the treatment. Application of a heavy dosage to sterilize the soil for a long time

TABLE 6

DOSAGE RECOMMENDATIONS FOR THE USE OF ARSENIC IN SOIL STERILIZATION ON CALIFORNIA SOILS

Soil			Toxicity group	Dosage,* As_2O_3† pounds per square rod
Texture	Type	Factors affecting arsenic availability		
Light and coarse	Sands and gravels, sandy and fine sandy loams	Normal	High	2–4
		Red or recent alluvial	Intermediate	4–8
Medium	Loams and silt loams	Normal	Intermediate	4–6
		Red or recent alluvial	Low	12
Heavy	Clay loams‡, clays, and adobe clays	Normal	Low	8–12
		Red or recent alluvial	Very low	12–20

* Values given in this table represent total dosages; this amount may be applied in several light treatments to meet the requirements of heavy soils or conditions of severe leaching.

† Since commercial sodium arsenite varies in As_2O_3 content, the weight of sodium arsenite required per square rod will depend upon the composition of the particular product being used.

‡ Clay loams from acid igneous rocks belong in the intermediate toxicity group and require a dosage of about 6 pounds per square rod.

would on first thought seem most economical. Where, however, leaching is severe (annual precipitation of 30 inches or more) or where the soil colloids cause low toxicity, losses of arsenic will be high, and the persistence of the treatment may not meet expectations. Under these latter conditions, a light annual application, though increasing the cost, minimizes losses from leaching and other causes. Evidently soils in the low-toxicity range require this type of treatment.

Leaching is an important factor in arsenic treatment of soils. Thus, Raynor found (*12*, p. 28–29) that the depth of penetration of sodium arsenite was influenced by the date of application since this was related to the rainfall. Under constant leaching, as in the banks of an unlined irrigation ditch, soil sterilization is not effective, all of the arsenic being removed by the seeping water. In regions of heavy rainfall, sterilization upon a given soil type is less permanent than in arid regions.

In connection with sterilization methods, arsenic trioxide (white arsenic) should be mentioned. This material, already used in plot studies (*5*), promises to become more popular when its special characteristics are better understood. Being relatively insoluble, it will lie in the soil for a year or more and gradually pass into solution, becoming tied up in high concentration in the top soil. According to experiments under central California conditions, one year is required to develop an effective toxicity in the soil. After the first year it is as effective as sodium arsenite, and because of its slow solution it lasts somewhat longer. This dry material, mixed with enough chlorate to give sterilization during the first year, should be the best reagent on heavy soils. Plot tests apparently bear out this conclusion (*5*).

In contrast to the retention of arsenic in the surface layer, common on heavy soils, a 12- to 20-inch penetration of sodium arsenite solution is common in light soils (*5*). This may be advantageous in controlling shallow-rooted perennials (*5, 12*). Consequently, the form of arsenic used should be related to the problem, and its varied behavior utilized to accomplish the ends in view.

The problems posed in the Introduction may be answered, at least in part, from the results of these studies. Concerning the type of arsenic compound to use in soil sterilization, the answer has already been indicated. For immediate results and for deep penetration on sandy soils from acid igneous rocks, sodium arsenite is preferable. In many other soils, especially heavy ones, red ones, and those from sedimentary rocks, decrease in toxicity is a serious factor; and to avoid excessive loss, either light annual application of sodium arsenite or the use of the less soluble trioxide seems advisable.

RECLAMATION OF ARSENIC-TREATED SOILS

From the soil-conservation standpoint, the slow accumulation of arsenic from compounds of low solubility to a toxic level in the soil is a serious problem (*11*). It means that a large reserve of insoluble arsenic is present, and that reduction in available arsenic must depend largely upon extensive leaching or upon the supplying of additional material capable of rendering arsenic unavailable. Though the use of iron oxides, or possibly red soils like Aiken clay loam, as soil amendments to reduce such toxicity offers an interesting field for research (*1*), nevertheless the slow solution of the residual, slightly soluble arsenic in the soil presents further difficulties. Apparently the continued use of arsenicals of low solubilty as insecticides on crops should be avoided, at least on light soils, for this method seems the best for providing lasting soil sterilization.

Where crop reduction follows the application of sodium arsenite, table 2 (p. 183) indicates that the toxicity may be greatly reduced over a period of time; and if the damage is not excessive, the soil may be reclaimed for agricultural use. But crop reduction following the continued use of slightly soluble arsenicals is a different matter; for the large reserve of arsenic present constitutes a supply capable of producing a long-continued toxicity. For reasons indicated above, even the use of soil amendments may not solve this problem. Where serious sterilization occurs, leaching would seem the only answer. Arsenic applications have not been effective below the water line in unlined irrigation ditches, and apparently the slow percolation of water will carry away almost any amount of arsenic in time.

SUMMARY

Biological tests show that arsenic toxicity is high in Fresno sandy loam, intermediate in Columbia fine sandy loam, and low in Yolo clay loam and Stockton adobe clay. Variation from previous tests may be explained by differences in the soil samples.

Repeated cropping shows that arsenic toxicity decreased in all 4 of these soils until, with the seventh crop, plants in the Yolo and Stockton soils survived in cultures containing 3,000 p.p.m. As_2O_3 in the air-dry soil. In the first crop test, no plants grew in cultures having more than 1,050 p.p.m. As_2O_3.

With repeated cropping, differences between Fresno and Columbia soils diminished. Though the limiting arsenic concentrations with the first crop were 280 and 550 p.p.m. As_2O_3, respectively, plants survived in cultures having 2,100 p.p.m. or more by the seventh cropping.

According to extensive tests involving short toxicity series in 80 California soils, arsenic toxicity can be correlated with texture, being high in sandy soils and low in clays. The most notable exceptions occur among the red soils, all of which, by rendering much arsenic unavailable, act like heavier types.

Arsenic sterilization on coarse, gritty soils in California requires a dosage of 2 pounds As_2O_3 per square rod.

Loams, silt loams, and those clay loams that are developed directly from acid igneous rocks or are highly weathered from other rocks require from 4 to 6 pounds per square rod.

Clays and adobe clays and some clay loams demand applications of from 8 to 12 pounds per square rod.

Red soils or recent alluvial soils from sedimentary rocks require approximately twice as much arsenic for a given type.

Light annual applications of soluble arsenic or use of dry arsenic trioxide with the addition of about 10 per cent sodium chlorate may be less wasteful on soils that render much arsenic unavailable.

Heavy leaching tends to reduce the concentration of available arsenic in the soil.

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ARSENIC FIXATION IN RELATION TO THE STERILIZATION OF SOILS WITH SODIUM ARSENITE

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ARSENIC FIXATION IN RELATION TO THE STERILIZATION OF SOILS WITH SODIUM ARSENITE[1, 2]

R. S. ROSENFELS[3] AND A. S. CRAFTS[4]

INTRODUCTION

DATA FROM GREENHOUSE EXPERIMENTS on the toxicity of arsenic to oats in 80 California soils are reported by Crafts and Rosenfels (*7*)[5] in another paper of this issue. In most of the soils tested, texture predominated as a determiner of toxicity; that is, toxicity was greatest in light and least in heavy soils. The few exceptions to this general rule are explained by the content of iron compounds of the soils (as indicated by their reddish color) or by the properties of the soil colloids. A similar relation between toxicity and soil texture has been noted by Cooper, *et al.* (*4* and *5*), Albert and Arndt (*2*), and Albert (*1*) working with South Carolina soils, and Reed and Sturgis (*11*), working with Louisiana soils.

The total arsenic content of a soil has not proved to be a satisfactory criterion of toxicity. As Vandecaveye, Horner, and Keaton (*15*) have shown, arsenic toxicity to barley is more closely correlated with the fraction soluble in 0.1 *N* ammonium acetate solution than with the fraction soluble in hot concentrated HNO_3. The results of Reed and Sturgis (*11*) show that the total arsenic content of the soil does not determine toxicity to rice. They indicate that toxicity is more closely correlated with arsenic soluble in 0.05 *N* HCl than with that soluble in water. According to Albert and Arndt (*2*), arsenic soluble in a collodion-bag dialysate is a reliable index of toxicity, whereas total arsenic is not. Greaves (*9*) has found no correlation between total and water-soluble arsenic in orchard soils.

Judging from other researches, not concerned directly with toxicity to plant growth, soils vary widely with respect to capacity for arsenic fixa-

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[2] This paper was made possible by the coöperative project on control of noxious weeds conducted by the California Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

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[5] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

tion[6] and retention against leaching. In the work of Schulz and Thompson (*13*), sodium arsenite was readily leached from a Wisconsin forest soil. McGeorge (*10*), on the other hand, showed by lysimeter experiments that sodium arsenite was strongly held despite excessive leaching by certain Hawaiian soils; he also found that soils varied in their capacity to fix soluble sodium arsenite. According to Dratschew (*8*) also, soils vary in capacity for arsenic adsorption. Stewart (*14*) records further evidence of variation between soils, showing that water extracts of various soils differ markedly in their capacity for dissolving lead arsenate.

The general principle may therefore be advanced that toxicity is directly related to the water-soluble, the dilute-acid-soluble, or some similar fraction of the total arsenic. This holds true whether one is dealing with the native arsenic in an untreated soil or with the arsenic applied as a soluble or an insoluble salt. Applied to the relation between toxicity and textural grade, this principle leads one to suppose that in heavy soils less arsenic is water-soluble for a given application, than in light soils. Since the ability of some soils to fix arsenic is greater than that of others, one may explain the relation of toxicity to textural grade by assuming a greater fixation of soluble arsenic in heavy soils than in light. In the present work, this assumption was submitted to experimental test; that is, an attempt was made to determine whether or not the toxicity of sodium arsenite in California soils, measured by greenhouse tests already reported (*7*), could be explained by arsenic fixation.

METHODS

The greenhouse technique in the determination of arsenic toxicity has been described in detail in the previous paper of this issue (*7*, p. 181). Results obtained by the method outlined are also given for 80 California soils (*7*, table 3) as yield of tops, in grams fresh weight, of oat plants corresponding to various applications of sodium arsenite to the soil expressed as p.p.m. As_2O_3 on the basis of air-dry soil.

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[6] In this paper the word *fixation* denotes the process of rendering soluble arsenic insoluble by contact with the soil. *Fixed arsenic* is *arsenic insoluble in water*. Both adsorption and chemical precipitation are included in this definition, the emphasis being placed entirely upon the extent of insolubility rather than the manner of its accomplishment.

This limited definition is necessary because the word *fixation* has had at least three different uses: first, it has been used to mean loss of availability to plants; second, loss of solubility; and third, retention against leaching. The present paper is concerned solely with the second of these meanings, and the previous paper (*7*) with the first. The term could therefore have been used in connection with either or both studies. To avoid ambiguity, the word was not used at all in the previous paper (*7*), and the specific definition given above adhered to in the present paper. This is purely a matter of convenience, and no claim is made that the "best" or most suitable use of the term has been attained.

Thirty-three of the 80 soils were chosen for the present study, a wide range of textural grades being selected. Toxicity curves were plotted for these 33 soils, the application of sodium arsenite expressed as p.p.m. As_2O_3 on the basis of oven-dry soil being plotted on the horizontal axis against the yield expressed as a percentage of the check, that is, of the yield without arsenic. The hygroscopic moisture content, which varied from about 0.5 to 8.0 per cent, was thus eliminated as a variable, and the curves all originated at the same height on the vertical axis. The data given in columns 4 and 5 of table 1 (p. 209) and in column 4 of table 2, (p. 213) were taken from the 33 curves thus produced.[7]

Arsenic fixation was measured in a series of standard laboratory runs conducted rigidly as follows:

Sodium arsenite stock solution was applied to samples of air-dry or moist soil equivalent to 100 grams of oven-dry soil. This solution, identical with the one used in the greenhouse tests, contained 5.00 grams As_2O_3 and 1.25 grams NaOH per liter. Enough water was added to make a total of 100 ml, including soil moisture and water added as sodium arsenite solution. Two samples of each of 6 different soils were handled in each run. One sample of each pair received a uniform application of arsenic; the other an application varying from soil to soil in a manner that will be explained.

The 12 soil and water mixtures thus prepared were agitated mechanically for 18 hours in 1-pint wide-mouthed jars by continuous rotation on the parallel shafts of a machine, and a portion of each was then filtered. Usually enough filtrate could be obtained under gravity, but sometimes filtration by suction was necessary. Some filtrates, especially those of sandy soils, were turbid. These were returned to the filter as often as necessary to get a clear solution. Occasionally some very fine suspended material remained despite these precautions.

The 1:1 extracts thus prepared were analyzed singly by the Gutzeit method (*3*, p. 306) within 2 hours of filtration,[8] without preliminary acid digestion or other treatment. An attempt was made to choose an aliquot for analysis that would yield approximately 0.015 mg As_2O_3, since this amount could be most accurately determined.

[7] These toxicity curves have not been published. Four examples of this type of curve are given in figure 5 (p. 226) of this paper, but the points plotted represent the means of 3 or 4 greenhouse tests. The toxicity data of tables 1 and 2 were taken from curves plotted from greenhouse results obtained simultaneously on 20 or 40 soils, each curve being derived from a triplicated test series on the soil concerned.

[8] According to tests, the arsenic content of extracts decreased markedly in some cases during periods of several days to a month. All extracts were therefore analyzed immediately after filtration. If the aliquot was poorly chosen, a new extract was prepared.

The directions for the Gutzeit method given by the Association of Official Agricultural Chemists (*3*, p. 306) were followed in detail with one exception: instead of maintaining the Gutzeit generator-bottle units at a constant temperature between 20° and 25° C for 1½ hours after addition of the zinc, the generator bottles were placed in an ice bath 10 minutes before the zinc was added and were held in it for 20 minutes after.[9] They were then removed and let stand at room temperature 1 hour before the stains were measured. Standard stains for 0.005, 0.010, 0.015, 0.020, and 0.025 mg As_2O_3 were prepared in duplicate for each run from the same Hanford-Pratt sensitized sheet used for the 12 soil-extract analyses. The paper sheets were sensitized by soaking 1 hour in 4 per cent mercuric bromide solution in 95 per cent alcohol.

All operations in the standard run described above were carried out in 2 days. The soil and water mixtures were customarily prepared in the pint jars in the afternoon and then agitated overnight. The filtrations were made the following morning, and the Gutzeit analyses completed the same day.

To calculate the percentage fixed, the difference between the concentration of arsenic applied and that found soluble was expressed as a percentage of the concentration applied. This value will be called the "apparent fixation," or "percentage fixed as determined."

Errors affecting the fixation values presented in this paper are as follows:

a) The inherent standard deviation of single analyses for arsenic by the Gutzeit method. This was determined by analyzing two different sodium arsenite stock solutions, of the composition given above, eighteen times each, and was found to be approximately ± 7 per cent for aliquots yielding 0.015 mg As_2O_3.

b) The effect of magnitude of fixation upon the accuracy of the fixation percentage. Since fixation was determined by difference in arsenic concentration of a solution before and after contact with the soil, the values for percentage fixed are subject to a sliding scale of error even though the standard deviation of the analysis itself remains constant. At a determined fixation of 0 per cent (the arsenic remaining 100 per cent soluble), the true fixation may lie anywhere between + 7 or —7 per cent; at 50 per cent fixed between 46.5 and 53.5; while at 100 per cent fixed the error is zero.

c) The effect of the soil extract upon the arsenic analysis. By attempting the recovery of 0.015 mg As_2O_3 in the presence of 1 ml or less[10] of 1:1 extract of several differerent soils, it was found that soil extract caused high results. Since the amount soluble as determined is thus too high, the perçentage fixed as determined is too low and must be given an upward correction to compensate for this source of error.

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[9] The use of an ice bath was recommended by the California State Department of Agriculture Division of Chemistry.

[10] In the routine analytical work the aliquots taken were restricted to no more than 1 ml and were sometimes as small as 0.06. All aliquots less than 1 ml were secured by measuring relatively large volumes of diluted extracts.

From this discussion it should be apparent that all values for percentage fixed determined by the method above outlined are subject to an uncertainty which diminishes in the direction of high fixation. The fixation values tend to be too low because of the presence of soil extract.

Figure 1 shows an attempt to illustrate graphically the range within which the corrected fixation values are expected to lie.

In this diagram, the 33 soils are grouped into two classes. One class includes the light soils which required 0.1 ml or less of extract[11] for an analysis. The tests on effect of extract show that the maximum error produced by any soil in this class was about + 9 per cent and the minimum + 3 per cent. An error of + 9 per cent combined

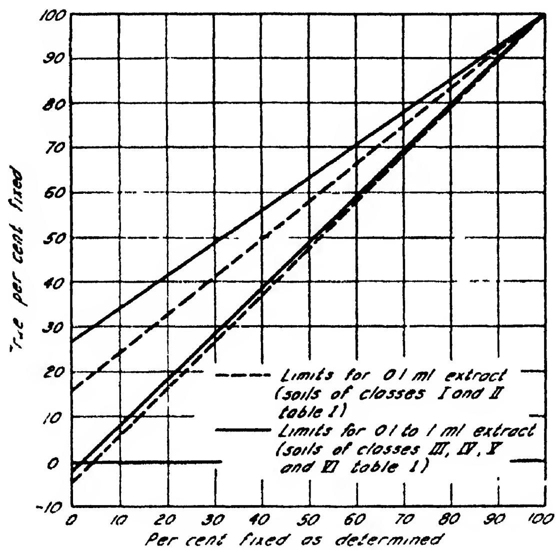

Fig. 1.—Apparent fixation, or percentage fixed as determined, plotted against the limits within which the true percentage fixed is expected to lie.

with + 7 per cent standard deviation gives a total of + 16 per cent. Negative corrections of 16 per cent in the *soluble* arsenic to compensate for this error would result in the series of *fixation* values given by the upper dotted line in figure 1. If the + 9 per cent error coincided with the negative value of the standard deviation, the net effect would be a + 2 per cent error. However, a lower limit based on corrections of —2 per cent is not plotted in figure 1 because only the limits for the entire group of soils are desired. Instead, the minimum error for the group of + 3 per cent is combined with a —7 per cent standard deviation for a net negative error of 4 per cent. Corrections of + 4 per cent in the soluble arsenic result in corresponding negative corrections in the fixation values; these are indicated by the lower dotted line in figure 1.

[11] As will become evident, the volume required depends on three factors: (*a*) application of arsenic; (*b*) time interval; (*c*) fixation. In the tests on effect of extract, the volumes chosen were those necessary to provide about 0.015 mg As_2O_3 with an application of 300 p.p.m. As_2O_3 and a time interval of 18 hours, these specifications applying to the data of table 1 (p. 209).

The limits indicated by solid lines apply to the balance of the soils, which require more than 0.1 and up to 1.0 ml of extract for an analysis. The maximum error in soluble arsenic due to extract in this group was + 20 per cent, the minimum + 5 per cent.

Statistical odds cannot be given for the expectation that the true values will lie between these limits. For soils midway between the extremes, with respect to effect of extract, the odds would be very high, and for soils near or at the extremes the odds would be lower.

RESULTS

Toxicity-Fixation Interrelation at 300 P.P.M. As_2O_3.—Table 1 presents data on toxicity, arsenic fixation, and soil moisture content at field capacity in 33 California soils. Of these soils, all but one, Yolo sand, are included in the group of 80 described in the preceding paper (*7*). Yolo sand is an infertile sand of practically no agricultural value deposited by the flood waters of Putah Creek.

The field capacities of the soils are given in column 3 of table 1.

The data on toxicity appearing in columns 4 and 5 were taken from the unpublished toxicity curves described in the section on "Methods." Column 4 gives for each soil the approximate application reducing the yield 95 per cent in the greenhouse test. Column 5 gives the area, in square centimeters, under each toxicity curve, measured with a planimeter. These areas are, of course, significant only on a relative basis, the absolute values being determined arbitrarily by the scales adopted in plotting the curves.

The data on arsenic fixation are given in columns 6 and 7. It was originally intended to compare the soils, as to percentage fixed, at a uniform application of 300 p.p.m. As_2O_3. The Gutzeit analyses of the sodium arsenite stock solution varied from run to run, however, and the actual applications were varied in an attempt to compensate for the supposed changes in concentration of the stock solution. It was learned later that these changes in concentration were not real, but were embraced by the inherent standard deviation of the analysis. As has been explained, the limits of error for the percentage fixed graphically presented in figure 1 include an allowance for the standard deviation.

In experiments referred to throughout the paper as the "first series," actual applications of sodium arsenite varied from 275 to 315 p.p.m. As_2O_3. To permit a valid comparison on the basis of 300 p.p.m. applied, slight corrections were made where necessary, by interpolation. As previously stated, different applications of arsenic were made to two samples of each soil. Each was plotted against the corresponding percentage fixed, and a straight line drawn between these points. The fixation percentage

TABLE 1

Soil Type, Arsenic Toxicity, and Arsenic Fixation at 18 Hours, in 33 California Soils

Soil characteristics			Greenhouse results		Fixation measurements at an application equivalent to 300 p.p.m. As_2O_3	
Soil grouping based upon toxicity and fixation of arsenic	Soil type, soil series, and textural grade	Field capacity (H_2O) on basis of dry soil	Application causing 95 per cent reduction in yield (As_2O_3 basis)	Area under curve	Apparent fixation	Limits of expected deviation
1	*2*	*3*	*4*	*5*	*6*	*7*
		per cent	*p.p.m.*	*sq. cm.*	*per cent*	*per cent*
Class I, toxicity limits 50 to 150 p.p.m. (col. *4*); fixation limits 20 to 45 per cent (col. *6*)	Greenfield coarse sandy loam	17.4	95	5.5	22.4	19.0–35.0
	Fresno sandy loam	17.6	65	5.3	27.5	24.5–39.0
	Hanford sandy loam	27.0	140	9.7	39.3	37.0–49.0
	Hanford fine sandy loam	17.8	140	10.7	42.3	40.0–51.5
	Average for class I	19.9	110	7.8	32.9	30.0–43.5
Class II, indeterminate between I and III	Delano fine sandy loam	18.4	250	16.0	35.9	33.0–46.0
	Rocklin sandy loam	21.0	450	22.0	48.8	46.5–57.0
	Rositas fine sand	17.4	100	8.9	55.6	53.5–63.0
Class III, toxicity limits 250 to 450 p.p.m. (col. *4*); fixation limits 60 to 85 per cent (col. *6*)	Sierra sandy loam*	17.1	275	21.5	43.6	42.5–58.5
	Chino silty clay loam	25.5	250	22.9	64.3	63.5–74.0
	Columbia fine sandy loam	23.1	250	20.3	65.7	65.0–75.0
	Yolo fine sandy loam	19.8	525†	31.6	67.3	66.5–76.0
	Madera loam	15.6	425	25.0	70.3	69.5–78.5
	Yolo silt loam	23.5	300	22.3	71.8	71.0–79.5
	Yolo sand	18.2	325	29.3	72.1	71.5–79.5
	Yolo loam	25.4	300	26.2	74.4	74.0–81.5
	Yolo clay loam	35.9	350	27.0	80.5	80.0–86.0
	Yolo adobe clay‡	36.1	375	24.3	86.0	85.5–90.0
	Average for class III	24.0	337	25.0	72.5§	72.0–80.0
Class IV, indeterminate between III and V	Fresno light clay	32.9	550	21.1	60.2	59.5–71.0
	Arbuckle clay loam	23.3	500	34.7	77.8	77.0–83.5
	Stockton adobe clay	42.4	700	34.7	80.3	80.0–85.5
	Montezuma adobe clay	36.8	425	40.6	86.0	85.5–90.0
Class V, toxicity limits 500 to 700 p.p.m. (col. *4*); fixation limits 85 to 93 per cent (col. *6*)	Madera clay	32.5	600	38.9	85.1	84.5–89.0
	Sites adobe clay	28.9	625	36.9	86.9	86.5–90.5
	Yolo clay¶	34.0	900¶	80.8	87.5	87.0–91.0
	Sacramento clay loam	40.0	575	35.3	88.5	88.0–91.5
	Egbert loam	40.9	525	36.1	90.1	90.0–93.0
	Panoche adobe clay	34.2	600	44.2	90.9	90.5–93.0
	Imperial clay	35.4	650	45.4	92.5	92.5–94.5
	Average for class V	35.1	639	45.4	88.8	88.5–92.0
Class VI, toxicity limits 800 to 1,300 p.p.m. (col. *4*); fixation limits 93 to 99 per cent (col. *6*)	Sierra gravelly loam‖	15.8	825	64.9	91.4	91.0–93.5
	Merced adobe clay	62.3	1,100	68.8	94.0	94.0–95.5
	Anita adobe clay	36.3	900	56.9	94.9	94.5–96.0
	Dublin adobe clay	46.2	850	77.8	95.5	95.5–96.5
	Aiken clay loam	29.2	1,300	95.0	98.6	98.5–99.0
	Average for class VI	38.0	995	72.7	94.9	94.5–96.0

* Shows a very low rate of fixation; at the end of 7 weeks is about equal to Yolo adobe clay.

† Repeat greenhouse test gave 275 p.p.m. as the application producing 95 per cent yield reduction.

‡ Shows a very high rate of fixation and consequently has too high a relative position at 18 hours.

§ Does not include Sierra sandy loam.

¶ This soil might belong in class VI. A different greenhouse test, however, gave 650 p.p.m. as the application producing 95 per cent yield reduction.

‖ Being in the same series as Sierra sandy loam, this soil has probably a low rate of fixation. If so, it would have too low a relative position at 18 hours.

at 300 p.p.m. was taken from this straight-line graph. Column 6 gives the percentages fixed, corrected to a uniform application of 300 p.p.m.; column 7, the limits of deviation within which the true values of these fixations may be expected to lie, these limits having been taken from figure 1. As previously indicated, two sets of limits are included in figure 1. One set applies to the fixation data for the soils in classes I and II, table 1. These soils, which fix the least arsenic, permit Gutzeit analyses on 0.1 ml or less of 1:1 extract at 18 hours with approximately 300 p.p.m. As_2O_3 applied. The other set of limits applies to the soils in the other four classes, which furnish 1:1 extracts such that 0.1 to 1 ml is required for an analysis with the same time and application.

The correlation of textural grade with fixing power, toxicity, and field capacity is indicated by the grouping of soils into classes given in column 1. The soils in class I—all light soils—exhibit the greatest toxicity, the lowest fixation, and the lowest field capacity. One can best see these facts by comparing class averages. Although the soils of class II do not fit into either I or III as delimited, they are obviously nearer these classes than any other. They have therefore been designated as "indeterminate" between I and III. The soils of class III illustrate the fact that textural grade is not the only factor involved in arsenic toxicity. This class includes the soils of intermediate toxicity, fixing power, and field capacity, yet includes 4 sandy soils and 1 adobe clay, 3 of which belong to the Yolo series. As the preceding paper (*7*) indicates, the Yolo series is a poor one for present purposes of illustration. This series is of recent alluvial origin from original sedimentary sources, and all types display a high capacity for arsenic fixation.

At this point, attention may be drawn to the importance of the time factor. As will become evident, soils do not all display the same rate of fixation. Thus both Sierra sandy loam and Yolo adobe clay are indeterminate in ultimate fixing power, but the former fixes much less arsenic than the latter at 18 hours.

Class IV is again indeterminate between adjacent classes. Class V includes what might be called medium-heavy soils, although there is little, in terms of soil type, to distinguish it from class VI, which includes the soils of lowest toxicity, highest fixation, and highest field capacity. Three outstanding exceptions appear in the last two classes. Egbert loam is an organic soil. Sierra gravelly loam and Aiken clay loam are both red soils and, as has frequently been observed, possess arsenic- and phosphorus-fixing capacities beyond expectation in terms of textural grade.

The sharply delimited classes given in table 1 represent, however, only one of many possible groupings. Broader class limits would eliminate

some of the seven indeterminate soils; but one need not suppose that fixation and toxicity should be correlated in every instance when one considers that, up to the attainment of fixation equilibrium in time, other sets of fixation figures at other time intervals would doubtless give different relative as well as absolute results. Furthermore, as will become evident (p. 225), the rate of change of percentage fixation with change in application, with a fixed time interval, differs somewhat for different soils. Thus, had the soils been compared as to fixing power at some application other than 300 p.p.m., somewhat different relative as well as absolute results would probably have been obtained.

There is also the question as to which measure of toxicity is best. In the preceding paper (7), the relative toxicity of arsenic in 80 soils was made evident by arranging the yield data in a regular order according to textural grade of the soil. The toxicity curves, previously described, in which percentage yield is plotted against application (on the basis of dry soil), provide a means of more accurate comparison. At least four methods, however, may be used to obtain a measure of toxicity from the curves. One may (1) determine the applications producing some given percentage reduction in yield; (2) measure the areas under the curves as expressing an average toxicity over the entire effective range of applications; (3) determine the percentage reductions in yield caused by some given application; or (4) measure the slopes of the curves at some fixed place. The class limits set in column 1 of table 1 were determined on the basis of the application causing a 95 per cent yield reduction. This criterion of toxicity was used because it is the most practical in terms of soil sterilization. Though a yield reduction of 100 per cent is, of course, the ideal, the application for this point cannot well be determined accurately because of the increasingly gentle slope of the curves as they approach the base line.

The area under the curve is apparently an inverse measure of average toxicity over the entire range of applications; and although this average toxicity might be of considerable theoretical interest in some cases, it has less practical importance than a determination of the application necessary to sterilize the soil.

Methods 3 and 4, mentioned above, were also tried. A determination of the yield reductions resulting from an application of 200 p.p.m., for example, provides a set of figures ranging from 100 per cent in some of the soils in class I to as low as 5 per cent in one of the soils in class VI. The determination of the slopes of the curves does not provide a generally satisfactory basis for estimating toxicity because the general trend of a curve may not be correctly indicated by a particular segment of it.

In most cases the two measures of toxicity given (table 1, cols. 4 and 5) substantially agree. In some cases they do not, however. Thus, with Fresno light clay and in similar cases, a disproportionately large application is required to accomplish the last 10 or 15 per cent of yield reduction necessary to the total of 95.

Finally, one must realize that the greenhouse runs could not all be made at the same time and that if the tests were repeated on all 33 soils, differences in relative results might occur. Repeated runs on several soils are reported in the previous paper (*7*).

Toxicity-Fixation Interrelation at Approximately 50 Per Cent Yield Reduction.—Besides the first series of applications at approximately 300 p.p.m., another set, hereafter called the "second series," was made in connection with the fixation studies. The actual applications are tabulated in column 3 of table 2, and the reduction in yield corresponding to each appears in column 4. The reductions recorded vary between 40 and 60 per cent. In seven cases where the reductions were beyond these limits, the data are omitted.

Column 5 gives the apparent fixation, or the percentage fixed as determined, at each of the recorded applications; column 6 the limits of deviation within which the true values are expected to lie. These limits (taken from fig. 1) were originally determined for applications of about 300 p.p.m. The applications in the second series were usually, however, less than this, and the average aliquot of extract necessary for an analysis was 0.74 ml as opposed to 0.33 ml for the first series of applications of 300 p.p.m. Despite this circumstance, the limits of deviation for applications of about 300 p.p.m. provide a generally satisfactory basis for evaluating the fixation data of table 2 because the limits were set to allow for extreme effects of extract. To illustrate, an upper limit of + 20 per cent was set for the error due to extract in all classes except I and II. The extracts of many of the soils in these classes, nevertheless, would not have produced so great an error even with much larger aliquots than were actually used.

An independent consideration of the relation between toxicity and fixation is afforded by the data of column 7. Here the concentrations of soluble arsenic at 100 per cent moisture, time interval 18 hours, are recorded for each soil at a series of applications giving approximately the same biological result (40 to 60 per cent reduction of yield). The class averages in this column reveal no clearly significant difference between any one class and another, which suggests that when fixation is allowed for, all soils are alike with respect to the concentration of soluble arsenic required for a given degree of toxicity. In interpreting these data, one

TABLE 2

ARSENIC FIXATION AT APPLICATIONS CAUSING YIELD REDUCTIONS OF APPROXIMATELY 50 PER CENT

Soil grouping	Soil type	Applications of arsenic (As_2O_3 basis), on basis of dry soil	Reduction in yield	Arsenic fixation measurements: As determined	Arsenic fixation measurements: Limits of expected deviation	Soluble arsenic (As_2O_3 basis) in 1:1 extract, limits of deviation
1	2	3	4	5	6	7
		p.p.m.	*per cent*	*per cent*	*per cent*	*p.p.m.*
Class I	Fresno sandy loam	25	41	40.0	37.5–49.5	15.6–12.6
	Hanford sandy loam	45	48	47.8	45.5–56.0	24.5–19.8
	Hanford fine sandy loam	57	48	56.9	55.0–64.0	25.6–20.5
	Average for class I	42	46	48.2	46.0–56.5	22.7–18.3
Class II	Delano fine sandy loam	95	58	52.8	51.0–60.5	46.5–37.5
	Rocklin sandy loam	72	41	64.7	63.0–70.5	26.6–21.2
	Rositas fine sand	55	50	55.5	53.5–63.0	25.6–20.3
Class III	Sierra sandy loam	115	46	56.5	55.5–68.0	51.2–36.8
	Chino silty clay loam	120	40	72.0	71.5–79.5	34.2–24.6
	Madera loam	155	53	79.0	78.5–85.0	33.3–23.2
	Yolo silt loam	105	40	78.4	78.0–84.5	23.1–16.3
	Yolo sand	137	40	82.5	82.0–87.5	24.7–17.1
	Yolo loam	137	40	82.0	81.5–87.0	25.3–17.8
	Average for class III	128	43	78.8*	78.0–84.5	28.2–19.8
Class IV	Fresno light clay	65	53	62.8	62.0–72.5	24.7–17.9
	Arbuckle clay loam	250	60	80.0	79.5–85.5	51.2–36.2
	Stockton adobe clay	130	45	89.5	89.0–92.5	14.3– 9.7
	Montezuma adobe clay	237	54	87.7	87.0–91.0	30.8–21.3
Class V	Madera clay	145	52	91.2	91.0–93.5	13.0– 9.4
	Sites adobe clay	155	45	92.7	92.5–94.5	11.6– 8.5
	Yolo clay	427	45	84.6	84.0–89.0	68.3–47.0
	Egbert loam	152	45	92.7	92.5–94.5	11.4– 8.4
	Panoche adobe clay	227	53	91.8	91.5–94.0	19.3–13.6
	Imperial clay	210	42	95.5	95.5–96.5	9.4– 7.3
	Average for class V	219	47	91.4	91.0–93.5	19.7–14.2
Class VI	Sierra gravelly loam	295	40	91.5	91.0–94.0	26.5–17.7
	Merced adobe clay	325	45	93.2	93.0–95.0	22.7–16.2
	Anita adobe clay	277	51	95.5	95.5–96.5	12.5– 9.7
	Dublin adobe clay	502	53	94.0	94.0–95.5	30.1–22.6
	Average for class VI	350	47	93.5	93.5–95.5	22.7–15.7

* Does not include Sierra sandy loam.

must again consider differences in rates of fixation; and since the toxic effect of the arsenic is being registered over a period of a month, one could not well specify a time interval that would be best from the biological standpoint. The effect of differences in moisture content at field capacity will be considered in a later section (see footnote 17, p. 225). At this point, suffice it to state that such differences apparently have less effect than might be expected.

The Time Factor in Fixation.—Table 3 gives the results of fixation measurements at approximately 7 weeks on samples of 9 of the 33 soils listed in table 1. Measurements on 4 of the same 9 samples at approximately 16 weeks, made in connection with another experiment (see fig. 3, p. 219), are also included. The soil samples used originated in standard greenhouse tests described on page 217 in connection with table 4. In the series from which the samples were obtained, each application of arsenic to the soil was replicated four times, and each can was watered daily. The cans were let stand in the greenhouse without watering for 2 days

TABLE 3

ARSENIC FIXATION AT 18 HOURS AND APPROXIMATELY 7 AND 16 WEEKS, AT AN APPLICATION EQUIVALENT TO 340 P.P.M. As_2O_3

Soil	Apparent fixation, 18 hours	Approximate 7-week interval		Approximate 16-week interval	
		Actual period	Apparent fixation*	Actual period	Apparent fixation
	per cent	*days*	*per cent*	*days*	*per cent*
Fresno sandy loam	25.7	46	72.4	105	74.5
Yolo adobe clay	85.0	54	89.4	116	90.4
Sierra sandy loam	40.8	54	87.9	119	93.3
Yolo clay	86.6	54	98.5	111	98.9
Greenfield coarse sandy loam	19.5	46	73.2		
Delano fine sandy loam	32.5	46	72.4		
Sacramento clay loam	87.6	49	99.8		
Egbert loam	89.7	49	99.9		
Sierra gravelly loam	91.0	54	99.4		

* The original data on soluble arsenic, from which these fixation percentages were computed, are given in table 5 for 4 soils. In table 5 the application of 340 p.p.m. is arbitrarily called "treatment 1."

after the oat plants were harvested at the end of the standard 30-day growth period. The contents of the 4 cans were then combined, thoroughly mixed, and stored in a sealed Mason jar. The application of sodium arsenite at a rate equivalent to 340[12] p.p.m. As_2O_3 was taken in all 9 cases. As moisture determinations showed, the sandy soils had fallen to about 50 per cent of field capacity, and the others had decreased to about 85 per cent. Fixation measurements were made according to the standard procedure previously outlined in the section on "Methods," the moist equivalent of 100 grams of dry soil being taken for each extraction. For comparison, the fixation percentages at 18 hours are also included. These were corrected to the basis of 340 p.p.m. As_2O_3 applied by the method previously outlined (p. 208) for correction to 300 p.p.m.

[12] Cultures were established in the greenhouse tests containing 0, 15, 40, 80, 140, 220, 340, 490, 680, and 920 p.p.m. As_2O_3, on the basis of oven-dry soil.

The errors in the measurement of arsenic fixation were somewhat greater than in the tests reported in tables 1 and 2. Limits of deviation for the fixation percentages cannot be given because the limits set forth in figure 1 apply only to aliquots of extract yielding approximately 0.015 mg As_2O_3. Because of the positive error arising from the presence of soil extract, all aliquots were restricted to a total of 1 ml regardless of concentration. As fixation increases with time, the amount of arsenic in the 1-ml aliquot often falls considerably below 0.015 mg As_2O_3, especially with heavy soils and small applications. In these cases the limits of deviation, if determined, would be somewhat farther apart than those given in figure 1.

Another possible source of error is the absorption of arsenic by the oat plants. Crafts and Kennedy (*6*) have shown, however, that the lethal concentration of arsenic in morning-glory is approximately 0.02 per cent As_2O_3 on the dry basis, in the tops, and 0.0003 in the roots (*6*, p. 339). With the higher of these figures as a basis of approximate calculation, and the highest yield recorded in the present cultures of oat plants (8.6 grams fresh weight), roughly 0.3 mg As_2O_3 would be absorbed if the root system weighed half as much as the tops, and the average content of dry matter were 10 per cent of the fresh weight. Since most of the cultures contained the moist equivalent of 500 grams of dry soil, about 0.6 p.p.m. As_2O_3 would be absorbed. The decrease in concentration of soluble arsenic would be less than this, however, due to replacement of part of the absorbed arsenic from the solid phase. Even though the arsenic tolerance of oats might be much greater than that of morning-glory, arsenic absorption would evidently not cause errors beyond the limits of error of the method itself.

Table 3 thus shows that arsenic fixation in Sierra sandy loam is approximately the same as in Yolo adobe clay at 7 and 16 weeks, although widely different at 18 hours. Yolo adobe clay shows a very slight increase in fixation after 18 hours, whereas Sierra sandy loam has apparently not accomplished all its ultimate fixation at the end of 54 days. Of the four soils studied at the longest interval, only Sierra sandy loam increased significantly in fixation after about 7 weeks.

Besides the results in table 3 other data on the effect of time are shown graphically in figure 2. In these experiments, the air-dry samples of soil were given enough total water to make 1:1 extracts at the start of the tests, as in the procedure for the 18-hour fixation tests. The arsenic application was equivalent to 250 p.p.m. As_2O_3 on the basis of oven-dry soil. The jars containing the arsenic-treated soils at 100 per cent moisture were let stand, with occasional shaking, at laboratory temperatures for

the intervals indicated, one jar for each point on each curve. The extracts were analyzed singly by the Gutzeit procedure as outlined. Comments in the preceding paragraph concerning limits of deviation for the fixation percentages apply here as well. The experiment was performed during June, July, and August, 1937. For comparison, the approximate 7-week

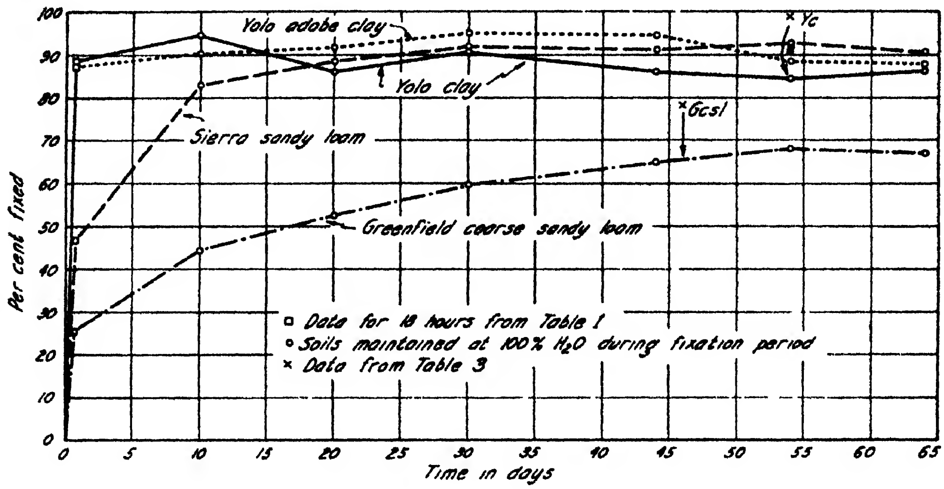

Fig. 2.—Time curves for Greenfield coarse sandy loam, Sierra sandy loam, Yolo adobe clay, and Yolo clay. All fixation percentages based upon an arsenic application of 250 p.p.m. As_2O_3 on the basis of dry soil.

fixation figures (table 3), corrected to a basis of 250 p.p.m. As_2O_3 applied, are plotted. Also the 18-hour figures, similarly corrected, are included as the first point on each curve.

These curves show that pronounced differences in rates of fixation do not appear after the first 10 days, even though differences in absolute level of fixation may exist throughout.

Whereas the 54-day points from table 3 for Sierra sandy loam and Yolo adobe clay lie fairly close to the curves, the 46-day point for the Greenfield coarse sandy loam appears to be significantly high, and the 54-day point for Yolo clay is unquestionably high at 99.2 per cent as opposed to 84.6 per cent on the curve. The points from table 3 represent fixation in soils held at field capacity or less, while the other points in figure 2 represent soils held at 100 per cent moisture. Apparently Greenfield coarse sandy loam and Yolo clay fix less arsenic from the 1:1 mixture than when held at a lower moisture content.

Relation of Moisture Content to Toxicity and to Arsenic Concentration.—Table 4 gives the results of standard greenhouse toxicity deter-

minations on 8 of the 9 soils[13] listed in table 3, along with the results obtained when the cans of soil were watered daily so as to maintain more nearly a constant percentage of moisture at field capacity. The tests were performed during February and March, 1937, after the original greenhouse work on arsenic toxicity in 80 California soils had been completed. They were intended to check the greenhouse results in the 9 soils selected, and also to measure the effect of the less-frequent watering practiced in the standard greenhouse runs. In the standard series, each application was duplicated, and the cans were watered whenever the plants showed

TABLE 4

GREENHOUSE DETERMINATIONS OF ARSENIC TOXICITY UNDER DAILY AND UNDER LESS-FREQUENT WATERING

Soils	Arsenic application (As_2O_3 basis) giving 95 per cent yield reduction	
	Watered daily	Watered 5 times during 30-day growth period
	p.p.m.	*p.p.m.*
Fresno sandy loam	135	135
Greenfield coarse sandy loam	200	200
Sierra sandy loam	200	150
Delano fine sandy loam	250	250
Yolo adobe clay	335	335
Yolo clay	650	800
Sacramento clay loam	900	900
Egbert loam	1,000	1,000

signs of wilting. This amounted to five times during the 30-day growth period. In the other series, each application was made in quadruplicate, and each can was brought to field capacity by weight each day.[14] As in the toxicity tests reported in table 1, curves were plotted relating the yield of tops, expressed as a percentage of the check, to the application of sodium arsenite as p.p.m. As_2O_3 on the basis of oven-dry soil. From these curves the applications causing a 95 per cent reduction in yield were determined as given in table 4.

Table 4 shows that in 6 cases out of 8 the results (toxicities) were identical under the two treatments, while in one case (Sierra sandy loam),

[13] Unfavorable physical condition of the soil made it impossible to get a uniform original stand of oats on Sierra gravelly loam. The results for this soil were therefore omitted as unreliable.

[14] The soil samples from two different applications for each soil in the series watered daily were saved at the end of the run, and utilized for the fixation measurements reported in table 3 at approximately 7 and 16 weeks.

the toxicity was higher with less frequent watering, and in another (Yolo clay) it was lower. Although the moisture content obviously fluctuated to some extent even in the cans watered daily, the fluctuation was much less than in the series watered only five times, wherein the moisture content undoubtedly fell to a point near the permanent wilting percentage between waterings. The fact that the toxicity was substantially unaffected by this marked decrease in moisture content suggests that the concentration of arsenic failed to increase as the moisture content diminished.

An attempt was made to secure experimental evidence on this point by determining the effect of varying the amount of water in the extract upon the concentration of soluble arsenic. Four soils of contrasting type were studied—Fresno sandy loam, Sierra sandy loam, Yolo adobe clay, and Yolo clay. The samples employed were the same ones used in the fixation tests at approximately 7 weeks reported in table 3. The extraction ratio tests were performed about 16 weeks after the arsenic was applied. During the period between these experiments, the soils were kept in sealed Mason jars at room temperatures. The tests were performed according to standard procedure, the moist equivalent of 100 grams of dry soil being taken for each extraction, and the extract being agitated 18 hours before being filtered and analyzed. The soil samples used were those originally given an application equivalent to 340 p.p.m. As_2O_3 (on the basis of oven-dry soil). Each experimental determination of concentration is plotted as a point in figure 3. The results with an extraction ratio of 1:1 (100 per cent water) were used to calculate the fixation percentages given in table 3 for approximately 16 weeks.

The curves of figure 3 were calculated in each case from the two experimental points marked by arrow; the adsorption equation used will be described in the next section, headed "Calculations." Each curve was extended to the moisture content at field capacity, but the lowest moisture contents reached experimentally were about twice field capacity.

For purposes of discussion, the following statement is given of the four general types of behavior which may occur when the moisture content of a soil is decreased.

1. The concentration of the given ion may rise in inverse proportion to the decreasing moisture content as, for example, with nitrate.

2. The concentration may rise but to a smaller extent than in 1. This is typical of ions which are adsorbed.

3. The concentration may remain practically constant. This is typically true with slightly soluble salts which provide a saturated solution over a wide range of moisture contents.

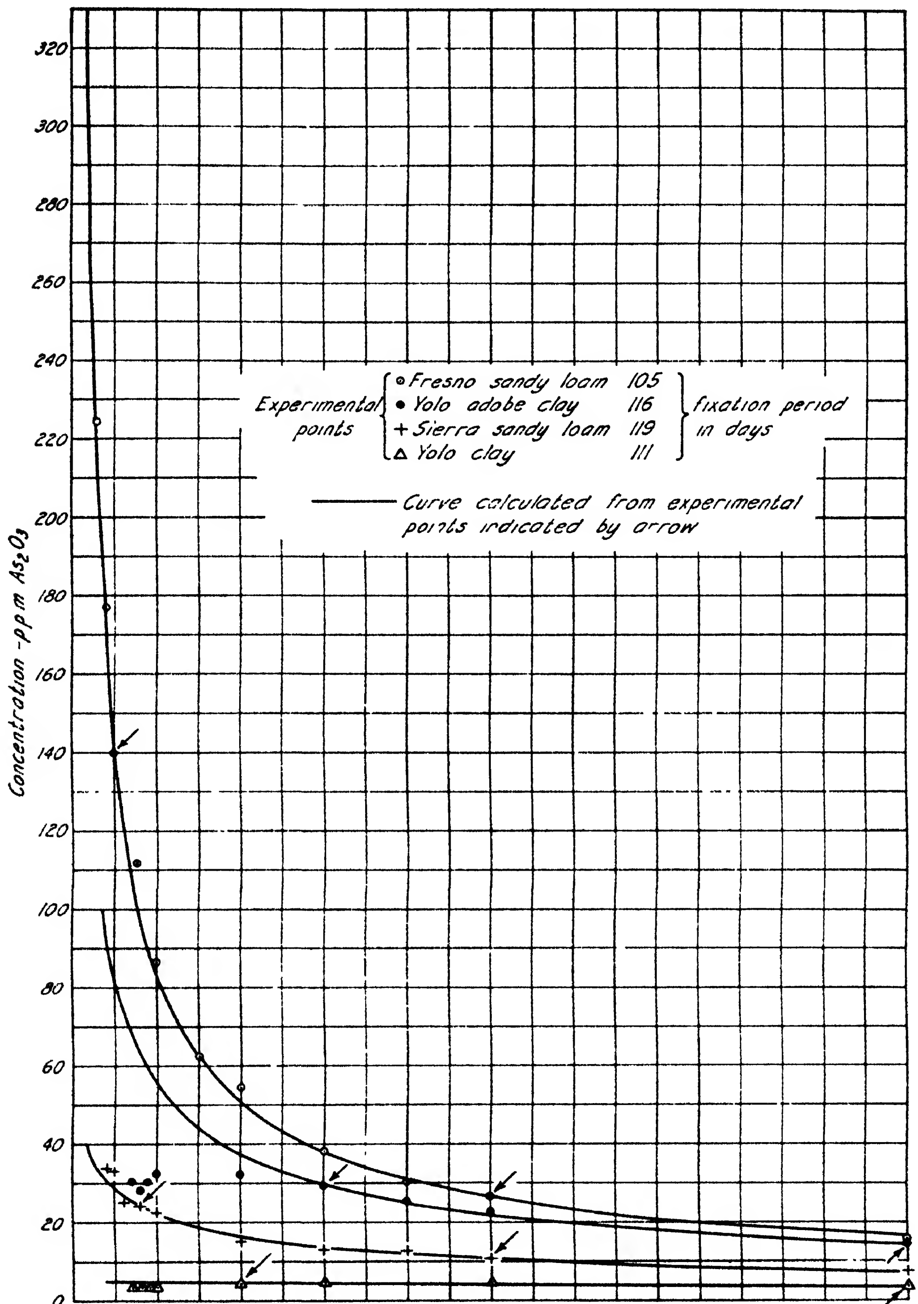

Fig. 3.—Concentration of soluble arsenic at various moisture contents for Fresno sandy loam, Sierra sandy loam, Yolo adobe clay, and Yolo clay. Application of sodium arsenite equivalent to 340 p.p.m. As_2O_3 on the basis of dry soil.

4. The concentration may decrease. This would happen in a mixture of slightly and readily soluble salts containing a common ion. For example, with calcium phosphate and calcium nitrate, a decrease in moisture content would increase the calcium ion concentration, and this in turn would result in a decrease in phosphate ion concentration owing to the operation of the solubility-product principle.

Behavior 1 is evidently not involved in the present study, but the other types are:

Fresno sandy loam and Sierra sandy loam constitute examples of behavior 2 over the range of moisture contents studied, as judged by the fact that the experimental points fall on or near the adsorption curves (fig. 3).

From 1,000 per cent to about 300 per cent water, Yolo adobe clay follows the adsorption curve but thereafter tends to follow first behavior 3 and then 4.

Yolo clay follows the adsorption curve fairly closely as plotted (fig. 3), but if a larger vertical scale were used, it would be seen that behavior 4 occurs between 200 per cent and 100 per cent water, and that behavior 3 apparently occurs between 100 per cent and 70 per cent. With this soil, however, the distinction between 2 and 3 is virtually nonexistent because the adsorption curve itself is practically a straight line with a very slight slope.

Returning to the toxicity data of table 4: clearly, with soils displaying behavior 3 or 4, no increase in toxicity would be expected as a result of a decrease in moisture. True, in table 4 the decreases in moisture content were in the range of field capacity or less, but if one may judge from the trends of the points in figure 3 for Yolo adobe clay and Yolo clay, an increase in arsenic concentration would not occur as the moisture content decreased. In the cases of Fresno and Sierra sandy loams, the application of 340 p.p.m. As_2O_3 is respectively beyond and at the upper limit of the sublethal range of applications. This is brought out in the toxicity curves of figure 5 (p. 226).

As will be shown in the next section in considering figure 4, the increase in concentration with decreasing moisture is far less than in figure 3 over the sublethal range of concentrations even though the behavior is dominantly one of adsorption. This principle is well illustrated by Yolo clay. With this soil, an application of 340 p.p.m. causes a reduction in yield of 63 per cent (fig. 5), which places this application in the sublethal range. The adsorption curve is so nearly flat that there would be no appreciable increase in concentration with a decrease in moisture even though adsorption were the dominant process.

CALCULATIONS

Curves relating the percentage yield to the concentration of arsenic in the soil solution would theoretically be alike for soils of different texture if fixation were the dominant factor in toxicity. Experimental data secured were not sufficient to permit an estimate of the concentration of soluble arsenic at each of the 9 applications used in the greenhouse tests. Given experimental measurements of concentration at 2 different applications, however, it is possible to calculate concentrations at other applications. The object of this section of the paper is to demonstrate these calculations, and to present curves of percentage yield against concentration of soluble arsenic resulting from them.

The equation[15] used was,

$$\log C = -\tfrac{3}{2} \log (F - x) + \log K,$$

wherein C signifies the concentration of soluble arsenic (p.p.m. As_2O_3), x the amount of arsenic in mg As_2O_3 fixed by 1,000 grams of dry soil, and F and K are constants. In the method of calculation used, F was first calculated from the following relations:

$$\log C_1 = -\tfrac{3}{2} \log (F - x_1) + \log K \text{ and}$$

$$\log C_2 = -\tfrac{3}{2} \log (F - x_2) + \log K. \text{ Subtracting:}$$

$$\log C_1 - \log C_2 = -\tfrac{3}{2} [\log (F - x_1) - \log (F - x_2)] \text{ and}$$

$$\log \frac{C_1}{C_2} = -\tfrac{3}{2} \log \frac{F - x_1}{F - x_2} \quad \text{and}$$

$$-\tfrac{2}{3} \log \frac{C_1}{C_2} = \log \frac{F - x_1}{F - x_2}. \text{ Therefore}$$

$$\log \frac{F - x_1}{F - x_2} = \tfrac{2}{3} (\log C_2 - \log C_1).$$

One can easily calculate the numerical value of the quantity, $\tfrac{2}{3} (\log C_2 - \log C_1)$, from the values of C obtained at the two different applications of arsenic made, letting C_2 be greater than C_1. If the antilog of $\tfrac{2}{3} (\log C_2 - \log C_1)$ be set equal to a, then

$$a (F - x_2) = F - x_1,$$

and from this,

$$F = \frac{a x_2 - x_1}{a - 1}.$$

Also,

$$F = x_2 + \frac{x_2 - x_1}{a - 1},$$

and the latter expression is more convenient to use.

Having measurements of C at 2 different applications, it is then possible to locate 2 points on, and hence determine, the straight-line curve of log C against log $(F - x)$.

[15] The writers extend thanks to Dr. Herbert S. Zuckerman, who suggested this equation and assisted with the calculations.

If 1:1 extracts are used, x is equal to $A-C$, where A is the application as mg As_2O_3 per 1,000 grams of dry soil. Once the curve is plotted, as many C, x pairs as desired may be obtained from it. If it is desired to determine A corresponding to various values of C, the general formula is:

$$A = x + \frac{CW}{1{,}000},$$

wherein W signifies the volume of water in ml associated with 1,000 grams of dry soil. Thus, in a 1:1 extract, W is equal to 1,000 and A is equal to $x + C$. Curves of A against C at any other moisture content may be easily obtained by substituting the desired value of W.

If measurements of C are available at 2 different moisture contents, A held constant as in figure 3, it is again possible to plot the straight-line curve of log C against log $(F-x)$. In this case

$$x = A - \frac{CW}{1{,}000}.$$

W corresponding to various values of C may be determined from the formula,

$$W = \frac{1{,}000\ (A - x)}{C}.$$

This is the method which was employed in constructing the adsorption curves of figure 3, the experimental C, W pairs used being marked by arrow. These points were chosen by inspection. The shape of the curves would be little affected by the particular pairs chosen except in the case of Yolo adobe clay. The determinations in the latter case reveal a change in trend below 300 per cent water; and the points marked by arrow were therefore chosen to fit the determinations down to this percentage.

Figure 4 gives the calculated curves[16] of C against A for the same four soils that were used in figure 3. All curves represent C at 100 per cent moisture ($W = 1{,}000$) except two of the curves for Fresno sandy loam, which were calculated to field capacity (17.6 per cent) and to the lowest extraction ratio reached experimentally in figure 3 (30 per cent). The calculations were based on the fixation data for approximately 7 weeks given in full for the 4 soils concerned in table 5. This table includes the original data from which the fixation percentages in table 3 at 340 p.p.m. were calculated. Applications listed under treatment 2 varied from soil to soil, being usually chosen as near as possible to the applications listed in table 2 (p. 213); the samples for these determinations were handled as described on page 214 in connection with table 3.

The justification for calculating these curves lies in the fact that the

[16] The sharp break in the curves at values of A of about 5 p.p.m. is due to the fact that, according to the equation, there is a low value of C for each soil at which x becomes equal to zero. At values of C below this point, x becomes negative. Since a negative x has no reality significance, the curves were continued to the origin as straight lines from the point at which $x = 0$. It was not determined experimentally whether this part of the curves is correct, but if incorrect, the effect would be to make the values of C slightly too high at low values of A.

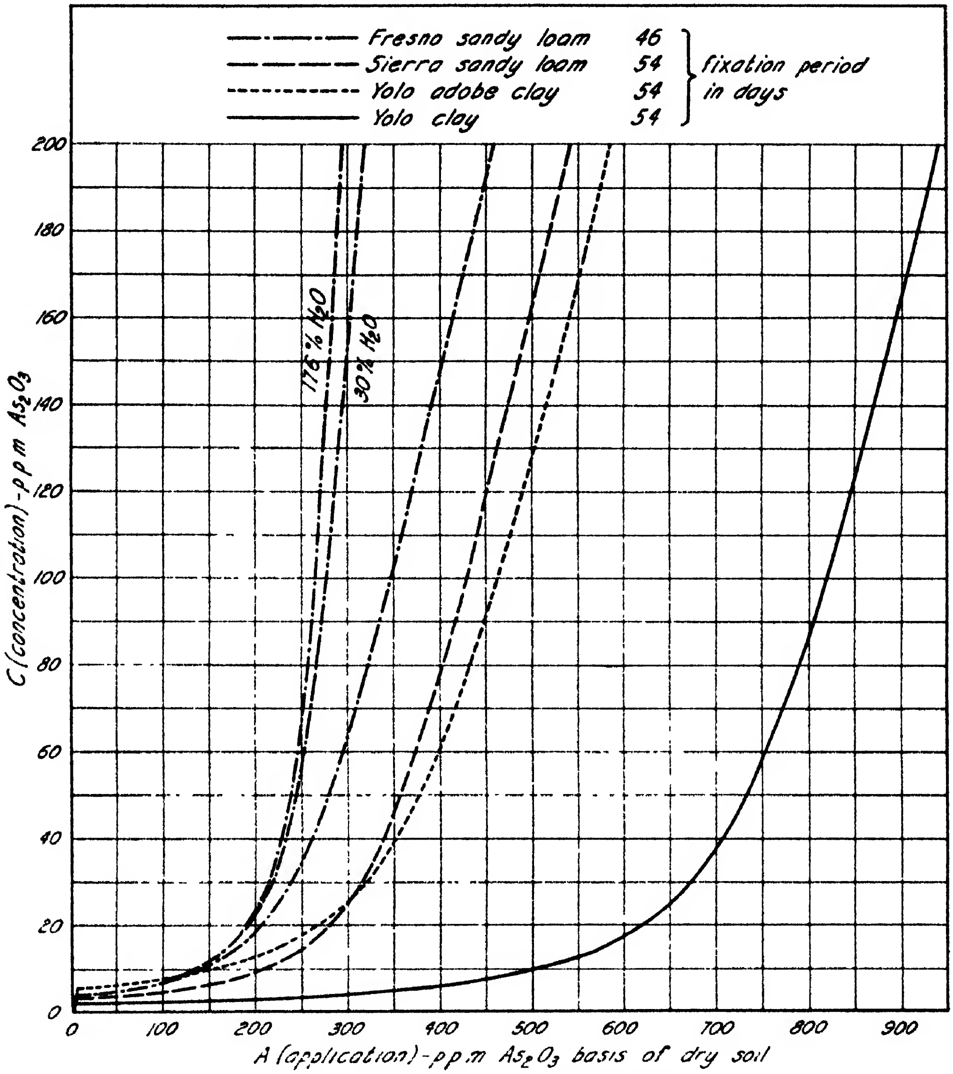

Fig. 4.—A (application) plotted against C (concentration) at 100 per cent water except as noted. Calculations based on fixation data at approximately 7 weeks given in table 5.

equation used was satisfactory in figure 3 wherever behavior 2 (adsorption) was involved. The fundamental relation is the change of x with C, and this can be tested either by varying A, the application, or W, the water content.

Regarding the time interval of approximately 7 weeks, it is clear that no interval could be defended as theoretically best, since the final greenhouse yields are related to the net effect of arsenic over a period of 30

days. The 7-week interval was chosen because, as figure 2 has indicated, a period of at least 10 days is preferable to 18 hours, and the 7-week interval was the next available. The latter, as is also evident from figure 2, gives about the same results as would be obtained at 30 days.

The values of C were calculated to 100 per cent water because this procedure was thought to give the best general approximation of the concentrations in the soil solution. Three points are involved: (a) with behavior 3 dominant, 100 per cent is best because the experimental measurements were made at this moisture content (1:1 extracts used); (b)

TABLE 5

VALUES OF C (CONCENTRATION) AND A (APPLICATION) AT APPROXIMATELY 7 WEEKS

Soil	Actual length of period	Application (As_2O_3 basis)		Concentration of As_2O_3 (C) 1:1 extract
		Treatment No.	Rate (A) on basis of dry soil	
	days		*p.p.m*	*p.p.m.*
Fresno sandy loam	46	1	340	94 0
		2	40	4 8
Sierra sandy loam	54	1	340	41 0
		2	140	6 2
Yolo adobe clay	54	1	340	36 0
		2	140	9 5
Yolo clay	54	1	340	5 1
		2	680	31 6

with behavior 4, the moisture content at which the experimental determinations were made is again the best, since the use of a lower value would involve a calculated increase in place of an actual decrease in concentration. If the decrease is not great, the values at 100 per cent water may be a close approximation to those in the soil solution. Yolo adobe clay is the only soil of the 4 studied in which behavior 4 might be of practical moment, and in this soil (fig. 3), the trend of the experimental points below 100 per cent water, while apparently downward, appears somewhat erratic; (c) with behavior 2, the increase in concentration in going below 100 per cent water is negligible over most of the range of sublethal applications. This point is well illustrated by Fresno sandy loam. The standard toxicity curve for this soil in figure 5 shows that only 3 per cent of the check yield is obtained at an application equivalent to 140 p.p.m. As_2O_3. Figure 4 shows that with A equal to 140 p.p.m., C is

equal to 10 p.p.m. at 100 per cent water, and 11 p.p.m. at both 30.0 and 17.6 per cent, the curves for the last-mentioned moisture contents having merged at a value for A of about 175 p.p.m. At all sublethal applications below 140 p.p.m., it evidently makes no practical difference which curve is taken. At an application of 220 p.p.m., however, there is still a 1 per cent yield (fig. 5), which places this application roughly at the upper end of the sublethal range, and at this point C increases from 24 to 35 in going from 100 per cent to field capacity. Thus, at the very lowest percentage yields, the values of C at 100 per cent water may be somewhat too low with soils showing behavior 2.[17]

Thus the curves of figure 4 apparently represent an approximation of the relation between the application, A, and the concentration, C, in the soil solution at the end of the greenhouse growth period. They may therefore be used to replot the toxicity curves of figure 5 on the basis of C, as above qualified, instead of A. Figure 6 gives the curves on this basis, the value of C corresponding to A for each percentage yield being taken from figure 4. Figures 5 and 6 are plotted on the same scale, and it is at once evident that the outstanding differences between the curves in figure 5 are absent in figure 6. This suggests that differences in fixation are largely responsible for the original differences between soils of different textural grades.

Although, as above noted, fixation appears to be the dominant factor in toxicity, attention may be drawn to the fact that the curves of figure 6 are not identical, a considerable divergence appearing between them below yields of approximately 30 per cent. This suggests the possibility that the concentration of water-soluble arsenic is not the only factor which may influence toxicity. If part of the insoluble or fixed arsenic were, for example, available in the case of the two sandy loams, the divergence between the curves would be largely accounted for. Part of the insoluble nutrient elements are of course known to be available in the soil, and it may be that because of replacement, less intense fixation, or other causes, some of the insoluble arsenic in certain soils may be effective biologically.

As mentioned on page 211, a comparison of soils as to fixing power at any given application is subject to the criticism that at other applications other relative as well as absolute results might prevail. This is illustrated in figure 4 by the crossing of the curves for Sierra sandy loam and Yolo

[17] Points *a*, *b*, and *c* above indicate that the concentrations given in column 7 of table 2 at 100 per cent water represent an approximation of the concentrations in the soil solution. Roughly equal toxicities are thus associated with roughly equal average concentrations for four different classes of soils, suggesting that the soils would be alike in field and greenhouse toxicity if it were not for differences in fixing power.

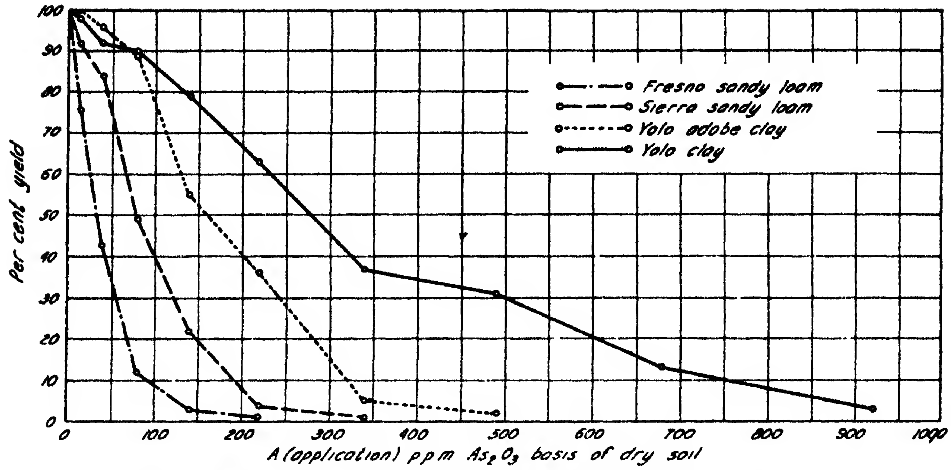

Fig. 5.—Percentage yields plotted against applications of arsenic to the soil for Fresno sandy loam, Sierra sandy loam, Yolo adobe clay, and Yolo clay, each point representing the mean of 3 or 4 greenhouse runs.

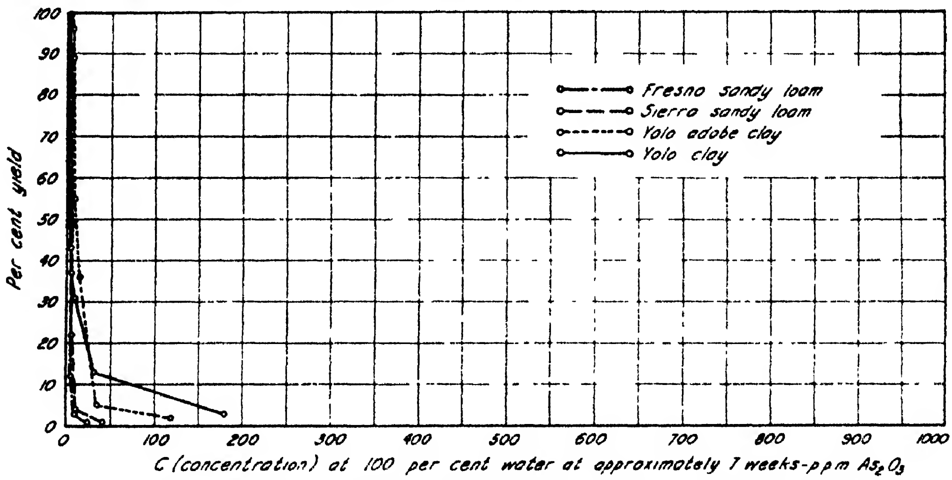

Fig. 6.—Same percentage yields as in figure 5 plotted against the calculated concentrations of arsenic at 100 per cent water taken from figure 4.

adobe clay at an application of about 305 p.p.m. Below this application, Sierra sandy loam would show the greatest percentage fixation, while above it the relation would be reversed, as shown in table 3 at approximately 7 weeks and an application of 340 p.p.m. Reversals in rank would undoubtedly occur in some cases in table 1 if comparisons were made at an application other than 300 p.p.m. Apparently, however, these reversals would involve minor shifts in position. Some of the present inconsistencies might be resolved and others accentuated, but apparently the broad relation between toxicity and fixation would be unaffected.

GENERAL DISCUSSION

The data presented in this paper lead to several general conclusions. First, different soils fix different percentages of a given application of arsenic. Second, the light soils of relatively low moisture-holding capacity, in general, fix least, and the heavy soils of high moisture-holding capacity fix most arsenic. Several exceptions are noted. Thus the red soils appear to have a high fixation capacity, apart from textural grade, because of their iron content, whether this be a chemical or physical or mixed effect. The one organic soil tested proved to have a high fixing power, though of medium textural grade. Third, as above qualified, light soils require less arsenic to sterilize them than do heavy soils. In a general sense, therefore, toxicity is inversely related to fixing power. Fourth, moisture content at field capacity is not important in determining toxicity. Thus the light soils display, on the whole, the greatest toxicity because they fix least arsenic, not because they hold little water. Fifth, considerable differences appear in the rates at which different soils fix arsenic.

With these broad conclusions in mind, one sees that the concentration of water-soluble arsenic serves as a general indicator of toxicity. As mentioned in the Introduction, other investigators (*15, 11, 2*) have suggested that the 0.1 *N* ammonium-acetate-soluble, the dilute-acid-soluble, and the dialyzable arsenic are each closely correlated with toxicity. Also (p. 225), as the present authors have indicated, part of the fixed or insoluble arsenic may be effective in causing toxicity. Judging from these observations, in the field of arsenic toxicity, as in the study of mineral nutrition, the question of "availability" is complex; in the present state of knowledge, one cannot state which fractions of the total arsenic should be considered strictly responsible for toxicity effects. The replacement of adsorbed phosphate by other anions occurs, as shown by Scarseth (*12*) and others; and Cooper, *et al.* (*4*) have observed in the field that arsenic toxicity is greatly increased by treatment with soluble phosphates. All that can be said is that toxicity is more closely correlated with water-soluble arsenic than with total arsenic, and that other fractions, more closely related to water-soluble than total, may also correlate with toxicity.

The data bearing on the effect of the amount of water upon concentration indicate that one should not use an unnecessarily large amount of water in making a soil extract for purposes of determining arsenic solubility if the results are to be reported on the basis of dry soil. Data at any convenient extraction ratio may serve certain purposes of comparison

within the limits of a given investigation, but the results obtained with 1:10 extracts by Reed and Sturgis (*11*), for example, are not comparable with those obtained with 1:5 extracts by Vandecaveye, Horner, and Keaton (*15*), or with those obtained with 1:1 extracts reported here, even though all are given on the basis of dry soil.

With behavior 2, 3, or 4, as described on pages 218–20, an erroneously high result will be obtained by multiplying the observed concentration by a factor equivalent to the extraction ratio, and the higher the ratio the greater the error.

SUMMARY

With certain exceptions, notably the red soils, light soils fix the lowest, and heavy soils the highest, percentage of a given application of sodium arsenite.

Light soils require the least and heavy soils the most arsenic to sterilize them. Toxicity is thus inversely proportional, in general, to arsenic fixation.

Arsenic fixation does not occur at a uniform rate in all soils, one soil showing an increase in fixation after 7 weeks, another showing very little after 18 hours.

Decreasing the moisture content of a soil below field capacity has no effect upon toxicity. This is apparently because, within the range of sublethal applications, the concentration of arsenic remains about the same in some soils and in others decreases.

Moisture content at field capacity is not an important factor in arsenic toxicity.

Curves of concentration, C, against application, A, may be constructed from two experimental determinations of concentration by the use of the equation

$$\log C = -\tfrac{3}{2} \log (F-x) + \log K.$$

Curves relating the percentage yield in the greenhouse tests to the concentration of soluble arsenic, plotted from the curves of C against A, were much more alike than the standard toxicity curves relating percentage yield to application of arsenic on the basis of dry soil—a fact suggesting that arsenic toxicity can be largely explained in terms of fixation.

ACKNOWLEDGMENTS

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TOXICITY STUDIES WITH SODIUM CHLORATE IN EIGHTY CALIFORNIA SOILS

A. S. CRAFTS

TOXICITY STUDIES WITH SODIUM CHLORATE IN EIGHTY CALIFORNIA SOILS[1]

A. S. CRAFTS[2]

INTRODUCTION

THE SUCCESSFUL USE of sodium chlorate as a herbicide in a region having such diverse soil and climatic conditions as California requires accurate knowledge of the relation of soil and climatic factors to its toxic action. Several publications have discussed the more important of these factors (*1–5, 8–9*)[3] and preliminary work on their relative importance has been reported (*3–5, 8–11, 13*).

For practical weed control with sodium chlorate, one needs a schedule of dosages to meet various field conditions. The principal difficulty in developing such a schedule is the many factors involved in the end result of chlorate application (*2*). Besides the initial toxicity[4] as determined primarily by nitrate concentration of the soil (*5*), leaching by rains and difference in susceptibility of weed species to chlorate are involved.

To solve the problems of chlorate toxicity, one must separate these several factors and determine the range through which each may be manipulated independently of the others. Only thus may all possible situations be anticipated and each factor properly adjusted. St. Johnswort (Klamath weed), for instance, on a sandy soil with an annual precipitation of 40 inches will require an entirely different treatment than hoary cress on clay soil in an arid region. In this field, obviously, the commercial concerns distributing sodium chlorate for herbicidal purposes have done little or nothing. Realizing the need for more accurate dosage recommendations, the writer collected 80 type soils of California, including most of the series important in agriculture. The effects of soil type and soil fertility upon chlorate toxicity in these soils were investigated. The relation between fertility and chlorate toxicity (*5*) revealed in these tests has been used as a basis for a proposed schedule of dosage that should prove useful wherever the chemical may be evenly distributed.

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[3] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

[4] The term "toxicity" has acquired a wide variety of meanings. For purposes of the present group of papers (*5, 7, 12*) the criterion adopted is the application of chemical causing an almost complete suppression of growth. This use of the word has developed because in the control of weeds the practical object is to inhibit development completely.

TABLE 1

TOXICITY OF SODIUM CHLORATE IN 4 CALIFORNIA SOILS, AS SHOWN BY GROWTH OF INDICATOR PLANTS*

Sodium chlorate expressed as p.p.m. $NaClO_3$ in air-dry soil	Yolo clay loam		Stockton adobe clay		Fresno sandy loam		Columbia fine sandy loam	
	Height	Fresh weight	Height	Fresh weight	Height	Fresh weight	Height	Fresh weight
Fifth run, harvested October 25, 1934								
p.p.m.	*cm*	*gm*	*cm*	*gm*	*cm*	*gm*	*cm*	*gm*
10	31	5.9	25	2.9	26	3.0	28	3.6
30	32	6.5	25	2.8	26	3.0	28	3.4
60	30	5.3	27	3.4	26	2.9	27	2.9
100	32	6.0	27	3.4	26	2.4	27	2.9
150	33	6.2	27	3.8	28	3.1	28	3.2
210	38	7.0	26	2.9	28	3.8	29	3.6
280	38	6.1	26	2.9	27	2.2	30	4.1
360	32	4.2	28	4.0	25	2.3	28	2.7
450	30	3.6	28	4.5	19	1.1	24	2.2
550	28	3.2	28	4.7	18	0.8	22	1.7
660	24	2.0	29	3.8	12	0.5	20	0.9
780	19	1.2	26	2.5	10	0.4	19	0.9
940	17	0.8	21	1.5	7	0.2	13	0.7
1,050	14	0.6	16	0.7	6	0.2	10	0.4
1,200	13	0.5	9	0.4	5	0.2	9	0.4
1,360	11	0.4	8	0.2	5	0.1	8	0.3
1,530	9	0.3	7	0.3	0	0.0	6	0.2
1,710	9	0.4	5	0.2	0	0.0	6	0.2
1,900	8	0.2	5	0.1	0	0.0	5	0.1
2,100	7	0.2	0	0.0	0	0.0	0	0.0
2,310	6	0.2	0	0.0	0	0.0	0	0.0
2,530	6	0.2	0	0.0	0	0.0	0	0.0
2,760	5	0.1	0	0.0	0	0.0	0	0.0
Check	30	5.7	25	3.1	24	2.5	24	2.6
Seventh run, harvested November 16, 1935								
p.p.m.	*cm*	*gm*	*cm*	*gm*	*cm*	*gm*	*cm*	*gm*
360	29	6.1	..	..	22	3.0		.
450	28	5.6		...	18	1.7	23	3.9
550	28	5.8	.	..	16	1.1	21	2.6
660	24	3.5			11	0.6	19	2.4
780	22	2.6	24	4.0	9	0.5	15	1.4
940	16	1.2	24	3.5	5	0.3	13	1.0
1,050	15	1.1	23	2.9	6	0.3	11	0.7
1,200	10	0.8	17	1.3	5	0.2	10	0.6
1,360	10	0.7	17	1.4	4	0.2	6	0.4
1,530	7	0.6	14	1.0	0	0.0	5	0.3
1,710	7	0.6	11	0.7	0	0.0	4	0.2
1,900	6	0.4	7	0.5	0	0.0	4	0.2
2,100	5	0.4	6	0.3	0	0.0	4	0.2
2,310	5	0.3	5	0.3	0	0.0	4	0.1
2,530	4	0.3	4	0.2	0	0.0	3	0.1
2,760	4	0.2	4	0.2	0	0.0	4	0.1
3,000	3	0.2	3	0.2	0	0.0	0	0.0
Check	23	3.9	18	2.6	21	2.9	21	3.3

* The check cultures represent the average of 20 replicates; all other values are the average of 5 replicates.

ADDITIONAL CROPS ON SOILS PREVIOUSLY TESTED

A previous publication (*4*) has discussed toxicity tests on 4 California soils, giving the data on the first 3 crops. This series was cropped four more times; and to complete the picture of the changes in toxicity that were revealed, table 1 has been prepared to show the results of the fifth and seventh crops. Values for the check cultures in this table represent the average of 20 replicates. All other values are the average of 5 replicates.

Considering all 7 runs, one sees that chlorate toxicity in the Stockton adobe clay, though highest at the beginning of the test, had dropped by the seventh run to a lower level than in the Yolo clay loam. The crop produced by the Stockton soil was, furthermore, consistently low. Although the initial toxicity seems related to the nitrate content of the soil (*5*), the loss in toxicity with time and cropping is caused by some soil factor apparently unrelated to fertility.

Toxicity in all 4 soils was lowered during these tests, and by the seventh run even that of the Fresno sandy loam had dropped to a value approximately that of the first run in the Yolo soil. By comparing points on the toxicity curve[5] for the first and seventh runs on these soils at the crop level of 1 gram, one finds the changes to be for Fresno sandy loam 150 to 560 p.p.m., or a difference of 410; for Columbia fine sandy loam 450 to 940 p.p.m., or a difference of 490; for Yolo clay loam 510 to 1,070 p.p.m., or a difference of 560; and for Stockton adobe clay 40 to 1,530 p.p.m., or a difference of 1,490. These are in the order of increasing clay content in these soils, but whether the changes are related to particle size or to some other property cannot be stated from these few tests.

EXPERIMENTAL METHODS ON EIGHTY SOILS

The soils and methods used in these tests have been described in detail in other papers (*4, 5*). The biological testing method developed through a series of stages from a simple technique involving single series of barley cultures in earthenware pots to a more carefully controlled practice with replication. One early concentration series is illustrated in figure 1. Concentrations of chlorate in these cultures, based on the air-dry weight of the soil, are 30, 120, 240, 375, 450, 600, and 900 p.p.m.

As these early tests soon showed the earthenware pots to be an uncontrolled factor, ordinary lacquered tin-plate cans were substituted. The No. 2 size of these cans holds 500 grams of most soils and is inexpensive

[5] Toxicity curves not published were constructed from table 1 and from table 9 of the earlier paper (*4*).

Fig. 1.—An early toxicity test with sodium chlorate in Yolo clay loam. Concentrations based on the air-dry soil are 30, 120, 240, 375, 450, 600, and 900 p.p.m. Barley was used as the indicator plant.

and convenient. Figure 2 shows a typical toxicity series in Ramada silt loam, an alluvial soil of intermediate texture and high fertility. In this series, concentrations from right to left are 0, 5, 15, 40, 80, 140, 220, 340, 490, and 680 p.p.m., based on the air-dry soil. This expanding series, developed after considerable experiment, has been used in the survey work reported in this and in an accompanying publication (*7*). As shown in the illustration, all tests were replicated three times.

Fig. 2.—A test series with sodium chlorate in Ramada silt loam. Concentrations, based on the air-dry soil, are 680, 490, 340, 220, 140, 80, 40, 15, 5, and 0 p.p.m. Three replicates.

TOXICITY RESULTS ON EIGHTY SOILS

During the development of the testing method the significance of results was considered. One of the first series set up in cans was a chlorate-toxicity test involving 15 concentrations and 2 checks, or 17 cultures per series. This test was replicated ten times and carried through 3 croppings. Data on the first crop (table 2) show how much variation may be expected between individual replicates at the various chlorate levels. The

average weight values in the last columns, if plotted, give a very smooth curve showing the relation between crop weight and chlorate concentration in the culture.

TABLE 2

TOXICITY OF SODIUM CHLORATE IN YOLO CLAY LOAM, AS SHOWN BY GROWTH OF INDICATOR PLANTS; 10 REPLICATIONS

(Harvested January 15, 1933)

Sodium chlorate expressed as p.p.m. $NaClO_3$ in air-dry soil	First replicate		Second replicate		Third replicate		Fourth replicate	
	Height	Fresh weight	Height	Fresh weight	Height	Fresh weight	Height	Fresh weight
p.p.m.	*cm*	*gm*	*cm*	*gm*	*cm*	*gm*	*cm*	*gm*
15	13.0	11.8	13.0	10.7	12.5	10.4	13.0	9.8
30	13.0	11.2	12.5	9.8	13.0	10.7	13.0	10.6
45	12.5	10.8	11.5	8.6	12.0	8.9	12.0	9.0
60	11.5	7.6	12.0	7.6	12.0	8.1	11.5	6.6
90	10.5	5.9	11.0	5.7	11.5	6.6	11.5	7.4
120	8.0	2.9	10.0	3.9	10.0	4.8	10.0	4.0
150	9.0	3.9	8.5	3.5	8.5	3.4	8.5	3.3
195	6.0	2.5	5.0	1.6	4.5	1.7	4.0	1.5
240	4.0	1.0	3.0	1.0	4.0	1.4	3.5	1.0
300	3.0	1.0	2.5	0.6	3.0	0.8	3.0	0.8
375	2.5	0.6	2.5	0.7	2.5	0.5	2.5	0.6
450	2.0	0.6	2.0	0.5	2.5	0.7	2.0	0.5
600	1.5	0.4	1.5	0.5	1.5	0.3	1.5	0.3
750	1.0	0.3	1.0	0.2	1.0	0.3	1.0	0.2
900	1.0	0.2	1.0	0.2	1.0	0.1	1.0	0.2
Check	13.0	10.5	13.0	10.1	12.0	10.3	13.5	11.5
Check	12.0	10.9	12.5	10.6	12.5	11.2	12.5	11.3

Sodium chlorate expressed as p.p.m. $NaClO_3$ in air-dry soil	Fifth replicate		Sixth replicate		Seventh replicate		Eighth replicate	
	Height	Fresh weight	Height	Fresh weight	Height	Fresh weight	Height	Fresh weight
p.p.m.	*cm*	*gm*	*cm*	*gm*	*cm*	*gm*	*cm*	*gm*
15	12.5	10.9	13.0	10.9	12.0	11.3	12.5	11.4
30	12.0	10.1	13.0	11.7	13.0	11.6	13.0	11.6
45	12.5	9.5	12.5	10.3	12.0	10.6	11.5	9.5
60	11.5	7.4	12.5	11.1	12.5	10.0	12.0	9.7
90	11.0	5.9	11.0	7.8	10.5	5.7	10.5	6.5
120	9.0	4.6	10.0	4.3	10.0	5.2	9.5	4.2
150	8.5	3.2	7.0	3.4	7.0	2.5	8.0	3.4
195	4.0	1.1	4.0	1.3	4.5	1.9	5.0	1.8
240	4.5	1.4	3.0	0.8	3.5	1.1	4.0	1.7
300	3.0	0.6	2.5	0.7	3.0	0.9	3.0	1.0
375	2.5	0.6	2.0	0.5	2.0	0.6	2.5	0.7
450	2.0	0.6	2.0	0.6	1.5	0.5	2.5	0.7
600	1.5	0.3	1.5	0.3	1.5	0.4	1.5	0.4
750	1.0	0.3	1.0	0.3	1.0	0.3	1.0	0.3
900	1.0	0.2	1.0	0.2	1.0	0.2	1.0	0.2
Check	13.0	11.0	13.0	11.4	13.5	11.7	13.0	11.2
Check	13.0	11.8	13.0	11.6	13.0	11.5	12.5	10.5

TABLE 2—(*Concluded*)

Sodium chlorate expressed as p.p.m. $NaClO_3$ in air-dry soil	Ninth replicate		Tenth replicate		Average		Average expressed as per cent of checks	
	Height	Fresh weight	Height	Fresh weight	Height	Fresh weight	Height	Fresh weight
p.p.m.	*cm*	*gm*	*cm*	*gm*	*cm*	*gm*	*per cent*	*per cent*
15	12.5	11.8	11.5	10.8	12.6	11.0	99.2	99.1
30	13 0	11.7	12.0	10.5	12.8	11.0	100.7	98.8
45	11.5	10.2	11.0	8.5	11.9	9.6	94.0	86.5
60	12.0	10.4	11.0	8.7	11 9	8.7	93.7	78.7
90	10.0	5.6	10.0	5.1	10.8	6.2	85.0	56.2
120	10.0	5.1	10.0	5.9	9.7	4.5	76.2	40.6
150	8.0	3.1	6.5	2.3	8 0	3 2	62.8	28.9
195	5.0	1.8	5.0	1.9	4 7	1 7	37.2	15.4
240	3.0	1.0	3.0	1.1	3 6	1 2	28.1	10 4
300	2.5	0.7	2.5	0.7	2.8	0.8	22.1	7.0
375	2.0	0 8	2.0	0.6	2.3	0.6	18.2	5.6
450	2.0	0.6	2.0	0.8	2.1	0.6	16.2	5.5
600	1 5	0 4	1.5	0 4	1 5	0.4	11.9	3.3
750	1.0	0.3	1.0	0.3	1.0	0.3	7.9	2.5
900	1 0	0.2	1 0	0.2	1 0	0 2	7.9	1.8
Check	12.5	11 3	12 0	12 1	12.8	11.1	101 5	100 3
Check	12.0	10 3	11 5	10.8	12.5	11 1	98 5	99 7

As too much work was involved in setting up and conducting tests involving this amount of replication, an attempt was made to reduce it by using only 5 replications. This arrangement was especially necessary because higher concentrations were needed if more than 1 cropping was to be used.

To ascertain the long-time behavior of arsenic, borax, and chlorate, series of tests using these chemicals in 4 California soils were established and have been reported—the borax results for 5 crops in a previous publication (*6*), the arsenic results for 7 crops in an accompanying paper (*7*), and the chlorate results in a previous paper (*4*) and in the present paper (table 1).

The early chlorate test indicated that crop production and toxicity were related, but with so few soils no generalization could be made. Consequently, chlorate tests were conducted on 80 type soils; and, with this number to judge from, the relation between high toxicity and low fertility became apparent. The soils used have been described in a companion paper (*7*). The technique was the same except that all chlorate tests included the 5 p.p.m. concentration and lacked that at 920 p.p.m.

Data on these tests, arranged in the order of increasing fertility as judged by crops on the untreated checks, are given in table 3. Where 2 or more series have the same crop weight in the checks, the ranking was determined by comparing total weights of the crops in the 3 check cul-

tures. In making up the averages reported, the figures in the second decimal place have been discarded, so that the averages lack the detail of the original totals. Where the totals were the same for 2 or more series, the soil with the highest toxicity, as indicated by the fewest surviving cultures, was placed first.

The relation of high toxicity to low fertility is apparent from table 3 and figure 3. That texture has no effect upon toxicity is evident. Without doubt, when other factors are constant, chlorate toxicity is largely determined by the fertility of the treated soil.

Among the apparent exceptions, No. 23, Rositas fine sand, No. 36, Superstition gravelly sand, No. 50, Meloland fine sandy loam, and No. 66, Imperial clay, attract attention. These soils are all from the Imperial Valley. Formed under arid conditions, they are high in salts, with sulfates, chlorides, and bicarbonates in abundance. Whereas nitrates are reported most effective in reducing chlorate absorption by plants, other anions are also involved; and these 4 soils apparently exemplify this fact. These anions hinder the absorption of chlorate and reduce toxicity without increasing crop production as does nitrate. No. 3, Dunnigan clay, and No. 10, Tulare clay, are 2 other soils moderately high in salts and very low in fertility. These 6 constitute the only serious exceptions to be noted in table 3.

The factors limiting the crop produced on a given sample of soil are numerous and varied. Besides fertility they include temperature, light, humidity, length of day, and combinations of these as related to the microbiological processes occurring in the culture. Since most of these factors were under little or no control in the greenhouse where the toxicity studies have been made, tests at different times and on different samples of the same soil vary appreciably. Table 4 presents data on tests at various dates on a number of soils. As in the accompanying paper (7) on arsenic, these results indicate the variations caused by lack of constant culture conditions and stress the desirability of running comparative tests on many soils at one time.

Since the relation between application and crop produced varies when tests are run under varying environmental conditions and when different samples of a given soil type are used, nitrate content or possibly total anion content might be a better criterion for judging chlorate dosage than is crop production. For practical purposes, however, the simple biological test is convenient where greenhouse facilities are available; and when comparisons are made simultaneously on all soils to be tested, the results seem reliable enough to determine relative dosages for application in the field.

TABLE 3

Toxicity of Sodium Chlorate in 80 California Soils as Shown by Growth of Indicator Plants

No.	Soil type	Date of harvest	Chlorate concentration—$NaClO_3$ in p.p.m. in air-dry soil: 0	5	15	40	80	140	220	340	490	680
			Fresh weight of plants									
			gm	gm	gm	gm	gm	gm	gm	gm	gm	gm
1	Aiken clay loam	Dec 26, 1935	0.5	0.3	0.1	0.1				*		
2	Pinole loam	Dec 26, 1935	1.2	1.3	0.5	0.1	0.1					*
3	Dunnigan clay	July 26, 1934	1.2	1.1	0.8	0.5	0.2	0.1				
4	Merced adobe clay	Dec. 26, 1935	1.4	1.5	1.3	0.3	0.1					
5	Conejo adobe clay	Dec. 26, 1935	1.4	1.3	0.5	0.1						*
6	Niland gravelly sand	May 15, 1935	1.4	1.2	0.6	0.5	0.3				*	
7	Aiken gravelly loam	Feb. 2, 1935	1.5	0.6	0.2	0.1			*			
8	Alamo adobe clay	Dec. 26, 1935	1.5	1.4	0.8	0.3	0.2				"	
9	Anita adobe clay	Dec. 26, 1935	1.6	1.5	0.6	0.1	0.1					
10	Tulare clay	Dec. 26, 1935	1.6	1.7	0.7	0.3	0.2	0.1				
11	Oakley sand	Dec. 26, 1935	1.7	0.9	0.4	0.1				*		
12	Corning gravelly loam	Feb. 1, 1935	1.7	1.3	0.4	0.1			*			
13	Fresno light clay	Dec. 26, 1935	1.7	1.0	0.5	0.1				*		
14	Yolo adobe clay	Dec. 26, 1935	1.8	1.2	0.4	0.2						
15	Tulare fine sandy loam	Dec. 26, 1935	1.8	1.2	0.6	0.2	0.1				*	
16	Tujunga sand	May 15, 1935	1.8	1.3	0.4	0.2	0.1				*	
17	Montezuma adobe clay	Dec. 26, 1935	1.8	1.5	0.5	0.2	0.1					
18	Clear Lake adobe clay	May 15, 1935	1.8	1.5	0.4	0.1	0.1					*
19	Farwell adobe clay	Dec. 26, 1935	1.8	1.4	0.6	0.3	0.1					*
20	Madera clay	Dec. 26, 1935	1.9	1.6	0.7	0.2	0.1					
21	Gridley loam	Dec. 26, 1935	1.9	1.6	0.8	0.4	0.1	0.1			*	
22	Landlow adobe clay	Dec. 26, 1935	1.9	2.0	1.5	0.5	0.2	0.1				
23	Rositas fine sand	May 15, 1935	2.0	1.8	1.0	0.8	0.4	0.2	0.2	0.1		
24	Sierra gravelly loam	Feb. 2, 1935	2.1	2.1	1.2	0.2	0.1	0.1			*	
25	Arbuckle clay loam	Aug. 19, 1934	2.1	1.9	1.0	0.3	0.2	0.1			*	
26	Panoche light loam	Dec. 26, 1935	2.1	1.7	0.7	0.3	0.1				*	
27	Diablo adobe clay	Dec. 26, 1935	2.2	2.0	1.2	0.4	0.2					
28	Stockton adobe clay	May 15, 1935	2.3	2.3	1.7	0.8	0.3	0.2	0.1			
29	Mariposa silt loam	Feb. 1, 1935	2.3	2.2	1.9	0.2	0.1	0.1				
30	Vina loam	Dec 26, 1935	2.4	2.1	1.7	0.6	0.2	0.1				*
31	Willows adobe clay	Feb. 2, 1935	2.5	1.8	1.0	0.2	0.1				*	
32	Oakdale coarse sandy loam	Dec. 26, 1935	2.5	2.3	2.0	0.7	0.2	0.1				*
33	Tehama loam	Dec 26, 1935	2.6	2.2	1.6	0.3	0.1				*	
34	Chino silty clay loam	May 17, 1935	2.6	2.3	1.3	0.7	0.4	0.2	0.1			
35	Holland loamy gravelly sand	Feb 1, 1935	2.6	2.0	1.5	0.4	0.2	0.1		*		
36	Superstition gravelly sand	May 17, 1935	2.6	2.3	1.5	1.0	0.8	0.6	0.3	0.1		*
37	Marvin silty clay loam	Dec. 26, 1935	2.7	2.7	2.4	0.8	0.3	0.1				
38	Sites adobe clay	Feb. 1, 1935	2.8	3.0	2.3	0.5	0.3	0.1			*	
39	Salinas clay	Dec 26, 1935	2.8	2.3	1.1	0.7	0.4	0.1				
40	Placentia light loam	May 15, 1935	2.8	2.4	1.4	0.8	0.4	0.1			*	
41	Foster fine sandy loam	Dec 26, 1935	3.0	2.3	1.4	0.7	0.2	0.1				
42	Greenfield coarse sandy loam	Dec. 26, 1935	3.0	2.7	1.1	0.4	0.1	0.1				*

* Germination of seeds prevented at this and all higher concentrations. Fresh weight of plants in cultures between reported weight and point of no germination was less than 0.1 gram.

TABLE 3—(*Concluded*)

No.	Soil type	Date of harvest	Chlorate concentration—$NaClO_3$ in p.p.m. in air-dry soil									
			0	5	15	40	80	140	220	340	490	680
			Fresh weight of plants									
			gm	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*
43	Porterville adobe clay	Dec. 26, 1935	3 0	2.8	1 9	0 7	0 4	0 1	0 1			
44	Rocklin sandy loam. .	Feb 2, 1935	3.0	2.8	2 0	0 4	0 2	0 1	0 1			*
45	Salinas fine sandy loam .	Dec. 26, 1935	3 0	2 9	2 4	0 5	0.3	0 1				
46	Hanford fine sandy loam	Dec. 26, 1935	3.1	2 4	1 3	0 7	0 4	0 2	0 1			
47	Montezuma adobe clay	May 15, 1935	3 1	3.1	2 6	0 9	0.4	0 2				*
48	Honcut loam	Dec. 26, 1935	3 1	3 2	3.0	1 4	0 4	0 2	0 1			
49	Redding gravelly loam.	Jan. 11, 1936	3 2	2 7	2 7	1 3	0 4	0 1				*
50	Meloland fine sandy loam .. .	May 17, 1935	3 2	3 3	3 2	2 4	2 1	1 6	1 1	0 9	0 5	0 4
51	Ramona sandy loam .	May 17, 1935	3 3	3 2	1.6	1 0	0 6	0 3	0 1		*	
52	Antioch clay loam..	Dec. 26, 1935	3 3	3.5	3 2	1 5	0 6	0 3	0 1			
53	Merced fine sandy loam	Dec. 26, 1935	3.6	3 6	2 4	0 8	0 3	0 2				
54	Yolo loam . .	Feb. 1, 1935	3 7	3 5	3 2	0 9	0 4	0 1			*	
55	Delano fine sandy loam	Dec 26, 1935	3 7	3 4	3 3	1 6	0 5	0 3	0 1			
56	Pleasanton loam	Dec 26, 1935	3 8	4 0	3 5	1 9	0 7	0 4	0.2			
57	Hanford sandy loam	Dec. 26, 1935	3.9	3 4	2 3	1 2	0 5	0 2	0 1	0 1		
58	Madera loam	May 17, 1935	3 9	3 8	3 3	1 4	0 4	0 1			*	
59	Yolo fine sandy loam	Feb. 1, 1935	3 9	4 1	3 9	1 0	0 5	0 1			*	
60	Fresno sandy loam	May 15, 1935	4 0	3 9	3 1	2 5	1 3	0 7	0 3	0 1		*
61	Yolo clay	Feb 1, 1935	4 1	4 0	3 9	1 3	0 5	0 1				
62	Arbuckle gravelly sandy loam	Feb 2, 1935	4 1	4 3	4 0	1 9	0 7	0 2	0 1	0 1		
63	San Joaquin loam	May 17, 1935	4 1	3 9	3 4	1 3	1 0	0 5	0 2	0 1		
64	Chualar fine sandy loam	Dec. 26, 1935	4 1	3 8	3 3	1 4	0 5	0 2				
65	Dublin adobe clay .	Dec. 26, 1935	4 1	3 5	2 4	1 1	0 7	0 4	0 2	0 1	0 1	*
66	Imperial clay	May 17, 1935	4 3	4 4	3 6	1 9	1 2	0 9	0 6	0 4	0 2	0 1
67	Sierra sandy loam	Feb. 2, 1935	4 4	4 6	3 9	1 6	0 4	0 1			*	
68	Capay adobe clay	Feb 1, 1935	4 5	4 7	4 5	0 9	0 2	0 1			*	
69	Esparto clay	Feb. 1, 1935	5 4	5 8	6 3	3 0	1 0	0 2	0 1			
70	Sites fine sandy loam	Feb. 2, 1935	5 6	6 1	5 6	3 8	1 5	0 4	0 1	0 1		*
71	Farwell loam	Dec. 26, 1935	5 6	5 5	5 3	3 2	1 2	0 3	0 1			
72	Ramada silt loam	Dec. 26, 1935	5 7	5 8	4 8	3 2	1 5	0 7	0 2	0 1		
73	Egbert loam	May 15, 1935	6 2	6 0	5 9	5 0	4 6	3 2	1 4	0 7	0 4	0 1
74	Columbia silty clay loam	May 17, 1935	6 5	6 8	7 3	5 7	4 0	2 1	1 2	0 7	0 5	0 2
75	Panoche adobe clay	Dec 26, 1935	6 9	6 2	5 5	4 0	2 5	1 2	0 8	0 4	0 3	0 1
76	Columbia fine sandy loam	May 15, 1935	7 8	7 5	7 8	6 0	3 8	2 6	1 8	1 1	0 7	0 3
77	Sacramento clay loam	May 15, 1935	7 9	7 3	7 7	6 1	3 2	2 1	0 9	0 5	0 2	0 1
78	Yolo silt loam	Feb 1, 1935	8 8	8 3	8 3	8 4	7 1	4 9	3 3	0 9	0 6	0 2
79	Yolo clay loam	Feb. 1, 1935	9 2	8 8	9 6	8 6	6 4	1 5	1 1	0.5	0 3	0 1
80	Yolo clay loam	May 15, 1935	11 2	12 0	11 8	9 4	7 1	4 7	2 9	1 4	0 8	0 4

* Germination of seeds prevented at this and all higher concentrations. Fresh weight of plants in cultures between reported weight and point of no germination was less than 0 1 gram

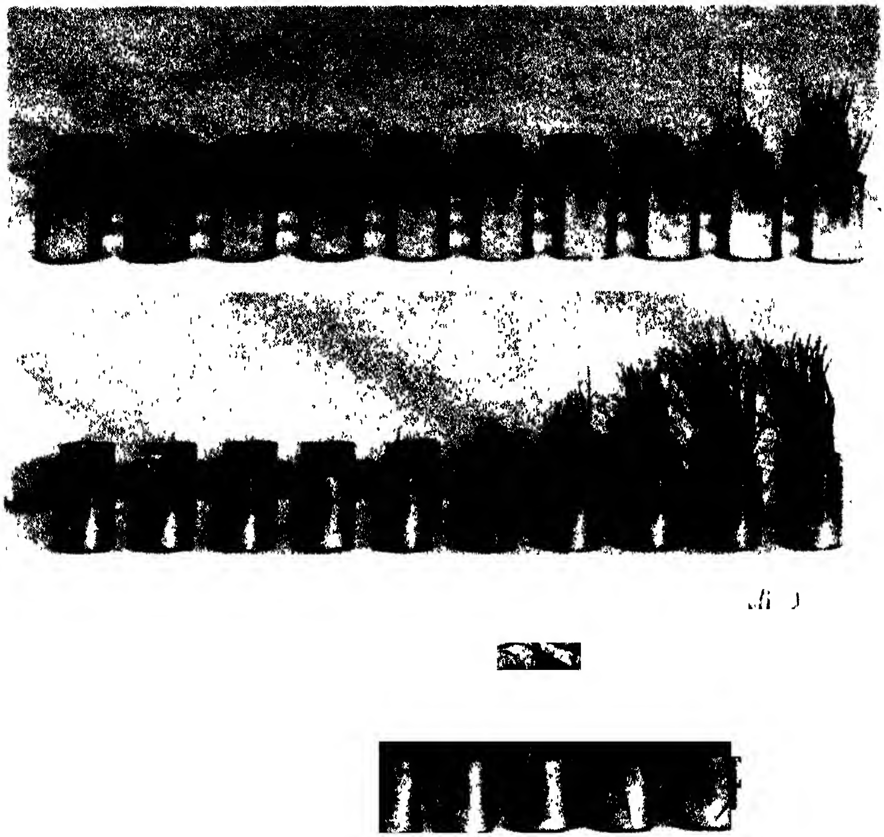

Fig. 3.—Test series with sodium chlorate in Aiken gravelly loam, Hanford sandy loam, and Panoche adobe clay. Concentrations run from 680 to 5 p.p.m. $NaClO_3$ with check cultures on the extreme right. Three replicates. Toxicity is closely correlated with fertility and is unaffected by texture.

DISCUSSION

To use sodium chlorate in weed control in semiarid regions one must know, among other things, the relative influence of its vertical distribution in the soil, and the comparative susceptibility of the plant species involved. Distribution, as previously explained (*2, 3, 4, 11*), depends largely upon leaching and is related both to precipitation and to soil type. Susceptibility in the practical sense includes: first, tolerance of the toxic action of the herbicide and, second, root distribution as related to penetration of the chemical into the soil.

How rainfall and soil type affect distribution of chlorate in the soil has been indicated (*2, 3, 4, 11*). With the extreme variations that occur in soil

TABLE 4

COMPARATIVE RESULTS OF TOXICITY TESTS ON REPEATED RUNS WITH SODIUM CHLORATE IN 6 CALIFORNIA SOILS

Soil type	Run No.	Date of harvest	Chlorate concentration—$NaClO_3$ in p.p.m. in air-dry soil									
			0	5	15	40	80	140	220	340	490	680
			Fresh weight of plants									
			gm	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*
Arbuckle clay loam	1*	Feb. 1, 1935	2.1	1.7	0.7	0.1	0.1					
	2†	Aug. 19, 1934	2.1	1.9	1.0	0.3	0.2	0.1				
	3‡	Dec. 4, 1936	3.6	3.4	2.3	0.4	0.1	0.1				
	4§	Feb. 17, 1936	4.4	4.8	6.6	0.9	0.5	0.1	0.1			
Columbia fine sandy loam	1†	May 15, 1935	7.8	7.5	7.8	6.0	3.8	2.6	1.8	1.1	0.7	0.3
	2‡	Nov. 26, 1936	8.8	9.2	9.1	8.1	5.2	2.2	0.7	0.1		
	3¶	Dec. 7, 1933	9.2	9.1	8.8	7.3	5.5	3.5	2.4	1.9	0.9	0.4
	4§	Feb. 17, 1936	11.4	11.0	10.4	8.8	6.5	4.4	4.1	1.0	0.6	0.1
Fresno sandy loam	1‡	Nov. 26, 1936	3.4	3.3	2.9	1.4	0.4	0.1	0.1			
	2†	May 15, 1935	4.0	3.9	3.1	2.5	1.3	0.7	0.3	0.1		
	3¶	Dec. 7, 1933	6.0	5.1	3.9	2.8	2.3	1.2	0.4	0.2	0.1	
	4§	Feb. 17, 1936	7.9	8.4	8.3	6.0	3.3	1.8	0.5			
Stockton adobe clay	1‡	Nov. 26, 1936	2.2	1.6	1.1	0.3	0.1					
	2¶	Dec. 7, 1933	2.3	2.2	1.9	1.0	0.5	0.1	0.1			
	3†	May 15, 1935	2.3	2.3	1.7	0.8	0.3	0.2	0.1			
	4§	Feb. 17, 1936	5.5	5.0	4.0	1.4	0.4	0.1	0.1			
Yolo fine sandy loam	1*	Aug. 19, 1934	3.6	3.0	1.7	0.7	0.4	0.2	0.1			
	2†	Feb. 1, 1935	3.9	4.1	3.9	1.0	0.5	0.1				
	3§	Feb. 17, 1936	5.6	5.6	4.8	4.9	3.2	1.1	0.1	0.1		
	4‡	Dec. 4, 1936	7.7	6.7	5.7	3.2	1.4	0.7	0.3	0.1		
Yolo clay loam	1†	Feb. 1, 1935	9.2	8.8	9.6	8.6	6.4	1.5	1.1	0.5	0.3	0.1
	2‡	Nov. 26, 1936	9.3	6.6	6.1	4.0	2.9	1.7	0.9	0.3	0.2	0.1
	3‖	Oct. 29, 1932	9.7	11.0	11.3	11.0	9.8	7.9	5.5	3.3	2.3	1.0
	4*	Aug. 19, 1934	11.0	10.1	8.9	6.7	4.4	2.6	1.2	0.7	0.3	0.2
	5¶	Dec. 7, 1933	11.1	10.7	9.5	7.8	6.0	4.6	3.8	1.8	1.2	0.2
	6**	Jan. 15, 1933	11.1	11.0	11.0	10.0	7.0	3.6	1.4	0.7	0.5	0.3
	7†	May 15, 1935	11.2	12.0	11.8	9.4	7.1	4.7	2.9	1.4	0.8	0.4
	8§	Feb. 17, 1936	17.0	17.4	16.5	11.3	9.1	4.9	2.6	0.9	0.3	0.1

* Repeat run standard check series.
† From table 3.
‡ Chemical nutrient series, from Crafts (*5*).
§ Nutrient series, from Crafts (*5*).
¶ By interpolation from Crafts (*4*, table 9).
‖ By interpolation from Crafts (*4*, table 1).
** By interpolation from table 2.

profiles and in the distribution of precipitation, exact recommendations are difficult to formulate, and local experience based upon empirical tests and field observation is essential to successful practice.

Plant susceptibility cannot easily be put on a comparative basis because it is hard to grow a wide variety of weed species simultaneously under constant culture conditions. Some work of this type has been done

(*3, 8, 13*), however, and more is contemplated. Collection of information is largely a matter of methods; and, as these are developed, all common weed species will be tested. The most valuable generalization coming from work thus far is that plants native to arid regions seem to tolerate more chlorate than plants of humid climates.

For any given plant, toxicity of chlorate seems to depend largely upon the numbers and kinds of anions in the culture medium. In leached soils, nitrate effects far overshadow those of other anions (*5*). In arid regions chlorides, sulfates, and bicarbonates enter the problem; and in these soils, probably, conductivity and nitrate content of soil extracts might be combined to provide a toxicity index. Perhaps, eventually, simple tests for nitrates and total salts will provide adequate information for formulating chlorate dosages.

Meanwhile an attempt will be made to draw up a schedule for the 80 soils that have been tested. At the outset, using field experience and the results of many plot tests, a basic scale of dosages adequate for controlling weeds that yield readily to chlorate is suggested. Such weeds are St. Johnswort, morning-glory, Russian knapweed, Canada thistle, and Johnson grass. Adequate penetration into the soil as determined by rainfall and soil type is assumed, and application should be made at such a time that decomposition of the chlorate is minimized. Under these conditions the soils of table 3, in which chlorate application up to 80 p.p.m. prohibited growth, should receive 1 pound per square rod. Those limiting growth at 140 p.p.m. should receive 2 pounds; at 220 p.p.m., 3 pounds; at 340 p.p.m., 4 pounds; at 490 p.p.m., 6 pounds; at 680 p.p.m., 8 pounds.

Under ideal conditions, of course, these dosages might be reduced even to one-half the values given. Under average conditions, this schedule is necessary. Against hoary cress, Bermuda grass, camel thorn, and white horse nettle this basic dosage should be doubled. Other common perennials range somewhere between these limits.

To workers versed in soil characteristics, one fact is apparent: though tests of the type in table 3 give a broad view of chlorate toxicity because this response is related to fertility, any generalized application of the dosage schedule may fail in many specific cases because fertility may vary so widely within a soil type. Numerous factors such as previous treatment, organic matter content, microflora, and the inevitable variations in deposition inherent in alluvial soils particularly, all tend to cause differences in fertility. Given such difficulties, one criterion may often prove useful in determining dosage in the field: the relative development of weeds or of crops in different localities at any given time is

often the best available measure of fertility, and a casual survey of plant growth always helps in determining application rate of chlorate.

The chlorate dosages necessary on certain soils and against some weeds are not only too expensive but harmful to the soils. When dosage exceeds 8 pounds per square rod, the cost approaches that of carbon bisulfide. On agricultural areas, considering the time lost through the residual sterility from chlorate and the undesirability of introducing sodium into the replaceable base complex, it seems advisable to use carbon bisulfide and return the land rapidly to crop production. But on waste areas, where permanence is to be desired, especially if deep-rooted perennials occur, chlorate is the logical herbicide in all cases.

As these studies emphasize, there are situations where chlorate is the best herbicide, other situations where carbon bisulfide is preferable and, as an accompanying paper (*7*) points out, some conditions favor the use of arsenic. One should not forget that in weed control numerous reagents have proved effective. In a comprehensive plan, all these should be used. The field operator should familiarize himself with the various methods and their limitations and should use each reagent to maximum advantage in the situation to which it is adapted.

SUMMARY

Repeated cropping of chlorate-treated soils resulted in continued loss of toxicity. Toxicity to the first crop (*4*) was highest in Stockton adobe clay, second in Fresno sandy loam, third in Columbia fine sandy loam, and lowest in Yolo clay loam. By the seventh crop toxicities had shifted so that Fresno sandy loam stood highest, Columbia fine sandy loam second, Yolo clay loam third, and Stockton adobe clay lowest. Although fertility largely governs the initial toxicity of chlorate in soils (*5*), some other factor controls the change in toxicity with time and cropping.

The toxicity-testing method used in studies reported here and in previous papers has been developed from a simple concentration series with barley in earthenware pots to the present technique using oats in replicated series in No. 2 cans. A test with 10 replicates gave excellent results but proved labor-consuming and slow.

Using a simplified technique with 10 concentrations replicated three times, 80 agricultural soils of California were tested for initial toxicity when treated with sodium chlorate.

The general relation of toxicity to fertility (*5*) was confirmed. In nearly every case, soils deviating markedly from the expected results proved to have come from arid regions and consequently to be high in total salts.

Repeated tests on a given soil type conducted at different times vary in the toxicities shown and reveal less correlation between toxicity and fertility than do the series run in large numbers for comparative purposes (table 3). For this reason, soils to be compared should be tested simultaneously.

Leaching and species susceptibility are known to affect chlorate toxicity. Under average field conditions a schedule of dosages of from 1 to 8 pounds per square rod should control susceptible species effectively, the dosages between these limits being fixed by the fertility of the soil. Under ideal conditions this schedule might be reduced. Under average conditions and against resistant species it should be doubled.

When chlorate dosage runs above 8 pounds per square rod, the cost approaches that of carbon bisulfide. Considering the loss of crops and the undesirability of introducing sodium into the replaceable base complex, carbon bisulfide seems preferable under these conditions.

Several chemicals, including arsenic, chlorate, and carbon bisulfide, have proved useful in weed control. In a comprehensive program all should be used, each under the conditions where it is most effective and economical.

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H I L G A R D I A

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HOST ORGANS ATTACKED BY BACTERIAL CANKER OF STONE FRUITS[1]

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INTRODUCTION

THE MOST DESTRUCTIVE and widespread phase of bacterial canker in *Prunus* is limb cankers. The organism also attacks leaves, blossoms, fruit, fruit stems, green shoots, and buds. In an earlier article (*1*),[4] comparatively little was said concerning the development of these last-named phases of the disease during epidemics. As later observations show, certain infected host organs are important, both from a crop loss standpoint, and from the standpoint of avenues through which the bacteria enter the tree. These infected organs are oversummering sources of the bacteria as well.

British workers are giving considerable attention to a bacteriosis of plums (*4*) and cherries (*5*). Since one of the organisms associated with this disease resembles that of the California disease,[5] the present paper also supplements earlier published (*1, 2, 3*) material.

Proof of the Pathogenicity of Cultures from Different Host Organs.—Isolations at different times and from material collected in different localities yielded a number of cultures. Inoculation and reisolation of certain of these cultures proved their pathogenicity to trees of plums, cherries, apricots, and peaches. In order, however to compare representative cultures from the various host organs more directly, extensive inoculations were made into cherry trees of the variety Bing. The results

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[3] Junior Plant Pathologist in the Experiment Station.

[4] Italic numbers in parentheses refer to "Literature Cited," at the end of this paper.

[5] The senior writer has demonstrated (*1, 3*) that *Phytomonas prunicola* (Wor.) Bergey *et al.*, cause of the disease in England, resembles *Phytomonas cerasi* (Griffin) Bergey *et al.*, the cause of bacterial canker in California. This evidence will not be reviewed here, nor will the status of *P. cerasi* as a separate species be examined. Suffice it to say that there are reasons for considering as identical *P. cerasi* and *P. syringae* (Van Hall) Bergey *et al.*, the latter causing lilac blight and citrus blast.

showed that organisms isolated from leaves, blossoms, blossom buds, fruit stems, fruit, and green terminal shoots produced cankers in every respect identical with those produced by *Phytomonas cerasi* from limb canker of plum (table 1).

TABLE 1

Pathogenicity of Organisms from Various Host Parts to Limbs of Bing Cherry, April, 1936

Culture number	Source of culture		Average length of cankers, in millimeters, 10 days after inoculation
	Organ and host	Locality	
1182*	Plum limb canker	Placer County	40
1996	Cherry leaf	San Joaquin County	35
1998	Cherry leaf	Sacramento County	53
1994	Cherry blossom	Sacramento County	59
1997	Cherry blossom	San Joaquin County	47
1999	Cherry blossom bud	Sacramento County	37
2000	Apricot blossom bud	Santa Clara County	35
2004	Plum blossom bud	Placer County	60
2005	Plum blossom bud	Placer County	56
2001	Cherry fruit stem	Sutter County	49
2002	Cherry fruit	Sutter County	63
2009	Plum terminal shoot	Placer County	70

* Original culture of *Phytomonas cerasi* derived from a single cell isolation.

RELATIVE IMPORTANCE OF THE DISEASE ON DIFFERENT ORGANS

Leaf Infection.—Sweet cherries have proved more subject to leaf infection (fig. 1, *A*) than other stone fruits. Although an occasional tree suffered severe defoliation, no extensive outbreak occurred. Aside from being a source for further leaf and fruit infection early in the growing season, this leaf phase did not contribute materially to the seriousness of the disease. With cherry bacteriosis, Wormald (*5*) regards leaf spots as important carryover sources during summer; but in California the dry and (in the interior) warm summers militate against successive waves of leaf and fruit infection that might occur in moister and cooler climates. It is true with this disease, as Wormald (*5*) maintains for his bacteriosis, that the leaf spots in early stages swarm with bacteria.

Blossom Infection.—Sweet cherries also proved most susceptible to blossom infection (fig. 1, *B*), though one rather severe outbreak was observed on apricot and though some varieties of plum are attacked at times. In 1935 blossom infection caused considerable direct loss of the cherry crop in three counties. This phase developed concurrently with serious outbreaks of limb and branch canker. In numerous cases infected

blossom clusters arising from primary and secondary limbs were foci from which the bacteria passed into such limbs, and thereby instituted the most serious phase of the disease.

The Duarte plum appears comparatively susceptible to attack through blossoms. During 1935 and 1937, when many Duarte trees were killed by the disease, blossom blight was common. It was not always clear whether

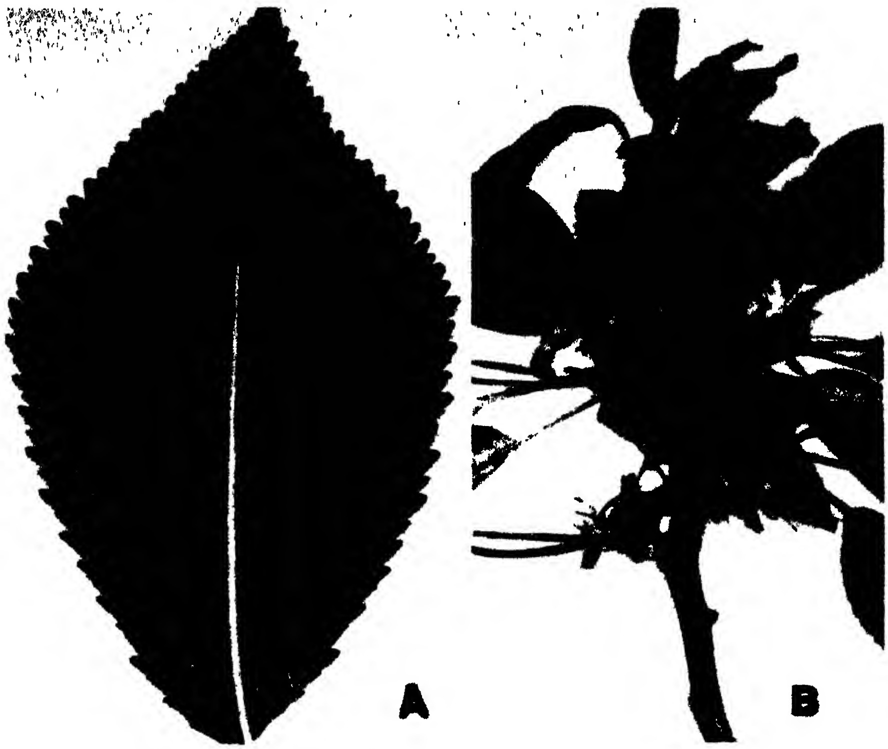

Fig. 1.—Cherry leaf and blossom infection by *Phytomonas cerasi*. *A*, Typical early stages of leaf-spotting of cherry. *B*, Blossom blight of cherry. The disease has proceeded into the spurs, blighting blossoms and leaves as well.

the bacteria had entered directly through the blossoms at the time the blossoms were emerging, or had entered between the scales of the winter buds. Wormald (*5*) was also unable to determine this point.

Bud Infection.—The winter buds (fig. 2, *B*), particularly those enclosing blossoms, were frequently infected. Sweet cherry, apricot, and certain plum varieties exhibited this type of infection more commonly than did peaches or almonds. Death of buds took place concurrently with severe branch and limb lesions of Duarte, Santa Rosa, and Wickson plums during 1935 and 1937. Bud infection contributed to the severity of the disease in three ways: (1) It caused considerable loss of blossom

buds. (2) It reduced the future fruitfulness of branches. In figure 2, *B* it may be noted all buds were killed for a considerable distance along a fruiting twig. This portion of the twig henceforth fails to produce either leaves or blossom. (3) If the infected buds were on spurs, they were frequently the seats whence the bacteria proceeded to invade the branches

Fig. 2.—Twig and bud infection by *Phytomonas cerasi*. *A*, Blighted terminal growth of Santa Rosa plum. *B*, Bud, spur, and blossom blight of cherry.

bearing the spurs. This was particularly true in Duarte plum; the death of many branches was traceable to cankers resulting from bud infection. In fact, the susceptibility to bud infection exhibited by the Duarte largely explains the high mortality of Duarte trees during 1935 and 1937. As a later article will show, once infection is established, the bark of Duarte plum is somewhat less favorable to extension of cankers than that of President, a variety suffering little loss in 1935 when it was not attacked through buds. In 1936 buds of President trees were attacked to

a considerable extent. This appeared to be clearly a case where severity of the disease was conditioned in no small way by the frequency of infection.

Figure 3, *C*, pictures an infected spur of cherry. Infection probably entered through a blossom bud and, by the time the photograph was made, had extended to the base of the spur. In such cases, especially if

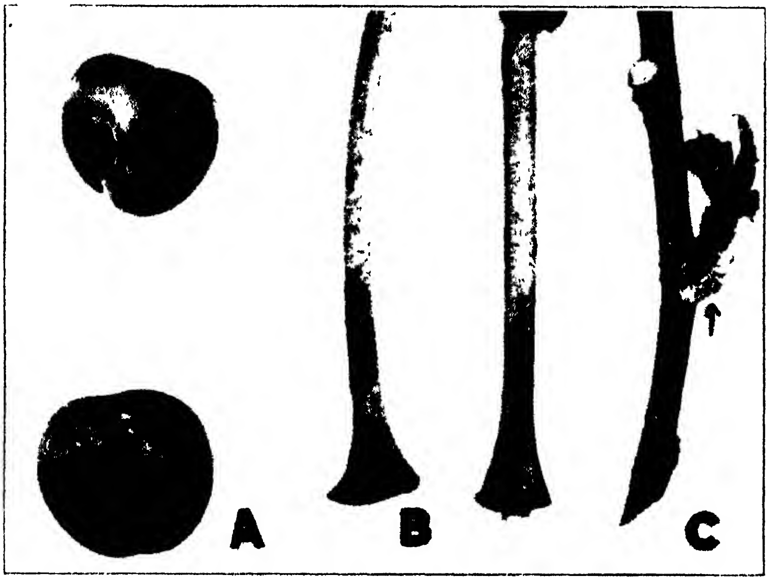

Fig. 3.—Cherry fruit, pedicel, and spur infection by *Phytomonas cerasi*. *A*, Lesions on fruit of Black Republican cherry. *B*, Lesions on fruit stems of Black Republican cherry. *C*, Blighted spur of Lambert cherry, showing gum (arrow) exuding from its base. Such spurs, particularly those on larger branches, are the foci whence the bacteria may spread through the bark tissue into the branch and thereby establish the more serious canker phase.

the disease extends into the branch, an oversummering source of infection is established. Leaf, fruit, and fruit-stem infection of cherries has been traced to such holdover sources. Infection of cherry buds also occurs with bacteriosis in England (*5*).

Green-Shoot Infection.—Infection of current terminal growth (fig. 2, *A*) though often noted in coastal counties, was found only once in the interior districts. In 1937 it developed concurrently with bud and branch infection of Santa Rosa, Wickson, and Duarte plums in the Sierra Nevada foothills. In no case was damage extensive. This green-shoot infection apparently does not play an important part as a carryover source, since the bacteria seemed not to survive the summer in the shoots to any great extent.

This type of infection is common with the bacteriosis of plums and cherries in England (*4, 5*).

Fruit and Fruit-Stem Infection.—One case of apricot fruit infection and one case of cherry fruit infection (fig. 3, *A*) have been reported. Considerable loss of crop was occasioned in the cherry orchard where black, sunken lesions bordered by water-soaked areas developed when the fruits were about half mature. Lesions on fruit stems (fig. 3, *B*) were also common, as was leaf spotting. The bacteria causing these infections had apparently come from infected buds and twig lesions.

Wormald (*4, 5*) reports fruit infection to be found on plums and cherries in England.

SUMMARY

The less common symptoms of bacterial canker occurring on leaves, blossoms, fruit, fruit stems, green shoots, and buds of *Prunus* spp. are discussed in their relation to severe outbreaks of the disease.

Organisms isolated from leaves, blossom buds, fruit stems, fruit and green terminal shoots of cherry, apricot, and plum produced cankers in every respect identical with those produced by *Phytomonas cerasi* from plum.

Leaf, fruit, fruit stem, and green-shoot infections have not contributed to the severity of epidemics. Infection of these organs commonly arises from bacteria originating in bud infections and twig lesions.

Blossom and particularly bud infections are frequently serious and develop concurrently with outbreaks of limb cankers. Such infections cause direct loss of crop, reduce future fruitfulness of branches, and produce foci from which the bacteria invade large limbs.

The symptoms pictured herein resemble those of a bacteriosis of cherries and plums in England. In other studies the causal bacteria of the two diseases have proved identical.

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FACTORS AFFECTING DEVELOPMENT OF THE BACTERIAL CANKER OF STONE FRUITS

EDWARD E. WILSON

FACTORS AFFECTING DEVELOPMENT OF THE BACTERIAL CANKER OF STONE FRUITS[1]

EDWARD E. WILSON[2]

BACTERIAL CANKER of *Prunus,* caused by *Phytomonas cerasi* (Griffin) Bergey *et al.*,[3] has been studied by the writer, with some interruptions, for the past seven years. Certain phases, including the serious limb-canker stage, have been described from time to time (*15–18*).[4]

This article discusses how various factors affect canker activity after infection is established.

SEQUENCE OF EVENTS IN DEVELOPMENT OF BACTERIAL CANKER UNDER ORCHARD CONDITIONS

Stages in the Activity of Cankers.—Although a general description of canker development is in print (*16*), later discussion will be clarified if the sequence of changes during canker activity is described in detail at this point.

An established canker will be taken as an example, and its development from early autumn to summer will be followed. This canker, present in the tree in early autumn, arose from an infection the previous winter or spring. It is a roughly elliptical area of dead bark, its margins grading away either into healthy tissue or, more frequently, into a series of reddish-brown streaks, which form a zone sometimes several inches wide at the apices of the necrotic center. In early autumn the canker appears inactive, the tissue involved being dry with no signs of water-soaking along the margins. The lateral margins of the canker may be delimited by a roll of new tissue, the outer layers of which are essentially callus in nature.

The nature of the reddish-brown streaks present at the apical margins is discussed in a later section. Nothing further will be said about them here except that they are called "dormant streaks" to distinguish them from the less noticeable, dull-brown, water-soaked streaks occurring at the margins of active cankers. The designations dormant and active are used because such streaks are characteristic, respectively, of dormant (or quiescent) and active cankers.

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[3] The status of *Phytomonas cerasi* as a separate species is in question (*15, 18*). Pending further work, however, the writer prefers to retain the species name.

[4] Italic numbers in parentheses refer to "Literature Cited," at the end of this paper.

Active streaks presumably are the path along which the bacteria advance. They are more numerous nearest the center of the canker. Thus the bacteria do not at first invade all the tissue along the advancing margins. Later, however, they do involve the tissue between those streaks nearest the center of the canker; but when the cankers stop extending in the summer, a wide zone of partially invaded tissue frequently remains at the upper ends of the necrotic area. This description, of course, is typical only of cankers in plums. In apricot and cherry the complete involvement of diseased tissue tends more often to keep pace with the advancing margins. Hence, as noted in an earlier article (*16*), cankers in these hosts often differ from cankers in the plum in being well-delimited, uniformly necrosed areas, with little partially invaded tissue at the margins. In the plums, on the other hand, the partially invaded zone, though not always easily distinguished from the healthy tissue while cankers are active, is generally present and noticeable after cankers become inactive. The varying appearance of this zone among different plum varieties is the subject for a later section.

With the gross characters of cankers in mind, the important landmarks of canker activity will be described. Activity of a previously inactive canker is noticeable because the margins of the uniformly dead center appear poorly defined and watery. In addition, the tissues between the dormant streaks partake of the same watery appearance. Next, the partially invaded margins of the cankers will begin to extend. This series of events is usually noticeable sometime in late October or in November.

Activity continues during midwinter; but invaded tissue may manifest the disease only by the presence of a few slightly brown active streaks. Sometime in early spring or late winter the tissue invaded during the winter rapidly turns darker brown and watery and develops a sour smell. Meanwhile the cankers will be seen to advance very rapidly in all directions, particularly upward and downward. Because the developments during this stage are rapid, a casual observer would believe that the cankers had originated and had spread throughout the bark of a tree in a period of only 3 or 4 weeks. In fact, growers who have seen this phenomenon frequently believe that disease development is confined entirely to spring and early summer.

The period of most rapid canker development may extend from about March 1 until late April. Thereafter there is a slowing-down and eventually an apparent cessation of extension. The first noticeable sign of this phenomenon appears in the uniformly necrosed margins. During activity the margins of the completely invaded tissue grade insensibly into healthy tissue, but when activity wanes they become well defined, par-

ticularly the lateral margins. The next sign of cessation of activity is in the streaks at the apices of cankers. These streaks, more or less diffuse during the active stage, begin also to take on well-defined margins and gradually change from a dull to a reddish brown. As shown later, the relative proportions of streaks eventually undergoing this change varies among cankers in the same tree and especially among cankers in trees of different varieties.

We have now traced canker development from a stage of quiescence in early autumn, through a stage of activity in late autumn, winter, and spring, back to a stage of quiescence in early summer. Throughout the summer, cankers remain inactive; or, at least, extension is so slow as to be undetectable. Not all, however, follow an identical course, for many fail to become active in the autumn. In one orchard of Duarte plums the percentage of cankers becoming active a second season varied from 30 to 65 in different years.

TABLE 1

OCCURRENCE OF BACTERIA IN DIFFERENT PARTS OF CANKERS

Areas of cankers from which isolations were made	Number of cankers studied	Number of isolations made	Per cent of isolations yielding bacteria
Zone of streaks at upper margins of inactive cankers	14	70	73
Zone of streaks at margins of active cankers	8	40	88
Recent uniformly necrosed tissue of active cankers	23	115	39
Uniformly necrosed margins of inactive cankers	17	85	24
Uniformly necrosed centers of inactive cankers	9	45	22

Relation of the Bacterial Population to Stages of Canker Activity.— Presumably the rise and fall of canker activity indicates corresponding fluctuations of bacterial activity in the diseased tissue. Freehand sections of diseased tissue in different stages of involvement have manifested tremendous differences in the bacterial population. According to one attempt at a semiquantitative determination, between late January and mid-February the number of bacteria increased 1,000 per cent. This increase occurred while diseased areas were undergoing rapid necrosis; the bacteria were invading all tissue of areas earlier only partially invaded. Apparently, furthermore, once the tissue is completely necrosed the bacteria decrease in numbers. Table 1 summarizes the results of isolations from different parts of cankers. In making the isolations, bits of diseased tissue were removed from five places in each part of the canker and were dropped in nutrient broth. Thus 70 isolations were made from the zone of streaks at upper margins of 14 inactive cankers. Of

these isolations 73 per cent yielded bacteria, whereas only 24 per cent of those from the uniformly necrosed margins of inactive cankers and a correspondingly low per cent of those from uniformly necrosed centers of inactive cankers yielded bacteria. Viable bacteria were obtained from 88 per cent of isolations from the zone of streaks at the margins of active cankers, but were found in only 39 per cent of isolations from recent uniformly necrosed tissue of active cankers. Not only have these results since been confirmed, but bacteria have been found as much as 2 inches beyond the visible apices of extremely active diseased areas. These findings agree with the earlier statement that the bacteria do not at first invade all tissue along the paths of advance.

Obviously, the foregoing series of isolations did not reveal the number of bacteria present, but did show the relative frequency with which viable organisms occurred in tissues differing in degrees of involvement. According to microscopic examinations, as well as the semiquantitative studies mentioned above, the peak of bacterial population is reached during the spring when the cankers are enlarging most rapidly; as activity wanes, so do the bacterial numbers. Judging from the infrequent occurrence of viable bacteria in margins of those cankers that have undergone uniform necrosis up to the visible limits of invasion, such cankers may fail to become active the following season. In cherry bacteriosis in England, Wormald (*20*) has noticed that cankers surrounded by a border of callus did not contain viable bacteria during July and August, whereas cankers not bordered by callus contained bacteria. Presumably, such cankers as did not yield bacteria in late summer would not again become active. Wormald neither mentions this possibility nor specifies at what period of the year naturally occurring cankers first become active under conditions in England. He does, however, say that inoculations produced cankers only when made after leaf fall in the autumn and that cankers did not increase in size after June. Regarding the plum disease in California, this much can be added: diseased areas that have entered the inactive state with little or no partially invaded tissue at the edge of their uniformly necrosed margins contain bacteria less frequently than do areas with a wide zone of such tissue at the margins. The apices of cankers of the former type are bordered by callus more frequently than those of the latter type. Hence, in a limited way, visible canker characteristics during the summer indicate the presence or absence of viable bacteria.

A somewhat similar situation exists with the fire blight disease. Opinions conflict regarding the frequency with which *Erwinia amylovora* (Bur.) Bergey *et al.* survives the winter in the different types of cankers,

designated by Tullis (*12*) as determinate and indeterminate. Brooks (*2*) and Miller (*7*) believe the indeterminate type to be the most frequent holdover source, but Rosen (*10*) does not concur.

EXPERIMENTAL EVIDENCE ON THE EXTENSION OF CANKERS DURING DIFFERENT SEASONS OF THE YEAR

To obtain evidence, beyond that published earlier (*16*), of the comparative rates at which cankers extend during different seasons of the year, inoculations were made at intervals throughout most of two years. Duarte plum trees were employed. Results of other, less systematic trials on President plum and Bing cherry do not differ essentially from those obtained in Duarte.

Methods.—The method of inoculating the bacteria into the trees was similar to that of most earlier experiments (*16, 18*): a sterile needle was passed tangentially through the bark, and a few drops of water suspension of the bacteria were injected into the holes with a hypodermic needle. Preliminary tests showed no particular necessity for sealing the inoculation holes during most of the year. From 50 to 70 inoculations were made into 5 trees at each time.

In securing data it was necessary to remove the outer bark, which rendered the canker useless for further measurements. Thus when a second set of measurements was required of the same series, 35 to 50 additional inoculations were made. Length and width of the diseased area in the bark were recorded. The width of the canker was not used in compiling the data, since it varied with the length of the tangential path of the needle through the bark. Sometimes the disease had extended along the cambium as a few narrow, brown streaks for a considerable distance beyond the limits of the bark lesion; but as these streaks varied along inoculations made in the same trees on the same day, they were disregarded in making measurements.

The data for comparative purposes were usually taken 20 days after inoculation and are expressed in millimeters as the average length of the cankers measured.

Results.—The first series of inoculations of 1934 made (fig. 1) on September 15 resulted in small lesions averaging about 5 millimeters long, and those resulting from October 20 inoculations were only slightly larger. The November 28 inoculations, however, produced cankers averaging 66 millimeters long. Canker extension, slow in mid-December, increased as spring approached, reaching a maximum on April 19. After this date, each succeeding inoculation produced progressively smaller

diseased areas until, by June 15, lesions of the same length as those developing in September were secured.

Inoculations begun on September 4, 1935 (fig. 2), were made at intervals until July 18, 1936. The symptoms produced in September, 1935, were more extensive than those produced in September, 1934, but mark-

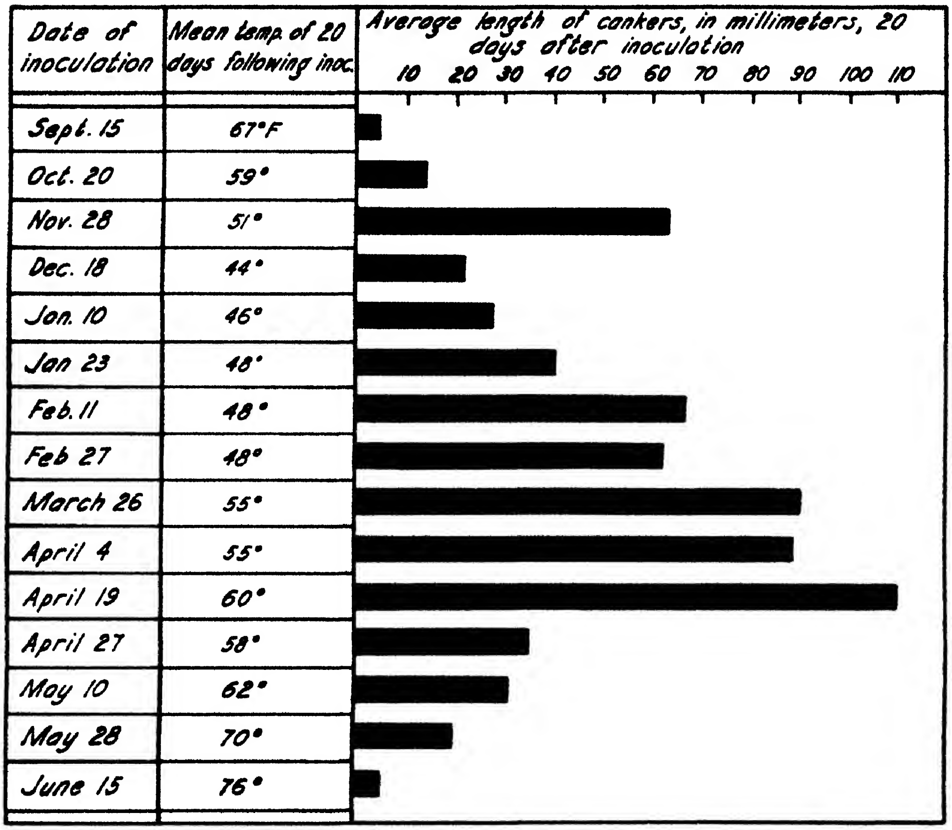

Fig. 1.—Development of bacterial canker from inoculations made at various times through the autumn, winter, and spring of 1934–35. The decline in the rate of canker extension as summer approaches, indicated by these data, parallels the observed cessation of activity in naturally occurring cankers. The slow canker expansion in midwinter is apparently the result of low temperature.

edly less extensive than those resulting from inoculation on October 8. From late October to mid-December results were variable, comparatively large cankers developing in all except the December 19 inoculations. During January and February the rate of canker extension increased, reaching a maximum in late February. The rate of extension was maintained at a relatively high level during March and April but began to wane in May. Lesions averaging less than 10 millimeters long resulted from inoculations June 5, July 1, and July 18.

To obtain further data on the comparative rate of elongation over the 20-day period, cankers produced by certain interval inoculations reported in figures 1 and 2 were measured at the end of 10 days and again at the end of 20 days. Since the cankers measured at the end of 10 days were necessarily destroyed in obtaining the data, a second set in the same inoculation series was measured at the end of 20 days. The averages obtained from these two sets of measurements appear in figures 3 and 4.

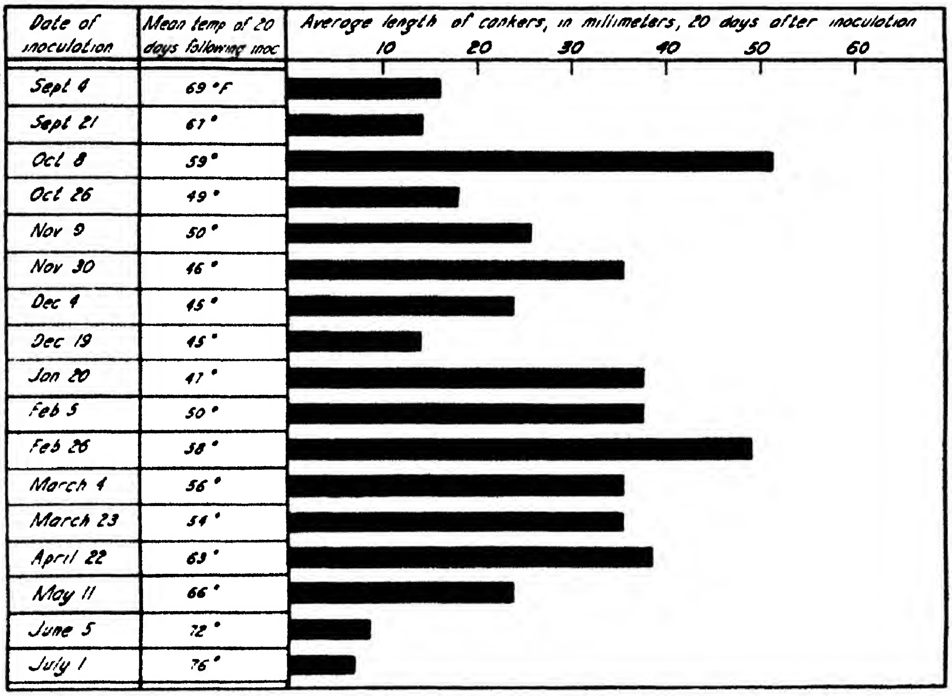

Fig. 2.—Development of bacterial canker from inoculations made at various times through the autumn, winter, and spring of 1935–36. Here again is noticed a decline in the rate of canker extension as summer approaches.

As figure 3 shows, cankers resulting from the January 10, 1935, inoculation had by January 20 extended an average of 15 millimeters and by January 30 had apparently extended an additional 15 millimeters. In other words, they had extended slowly but uniformly during the 20 days. We note, furthermore, in the successive inoculations made between January 10 and April 19, a progressive increase in the length the cankers attained during the 20 days after inoculation, but a progressive decrease in the increment of growth during the last 10 days of the period. Those resulting from April 19 inoculations apparently extended rapidly for 10 days or less and then stopped, or extended so slowly as to be undetectable in the measurement data. Likewise, cankers produced by the

May 10 inoculation extended for 10 days or less, though less rapidly than those produced by April 19 inoculations, and then stopped.

The data secured in 1935–36 (fig. 4) agreed in general with those of the former year. Inoculations before October 8 produced small cankers, which apparently extended during the first 10-day period after inoculation and then stopped. On the other hand, inoculations between October 8 and March 4 produced in 10 days cankers of somewhat greater length,

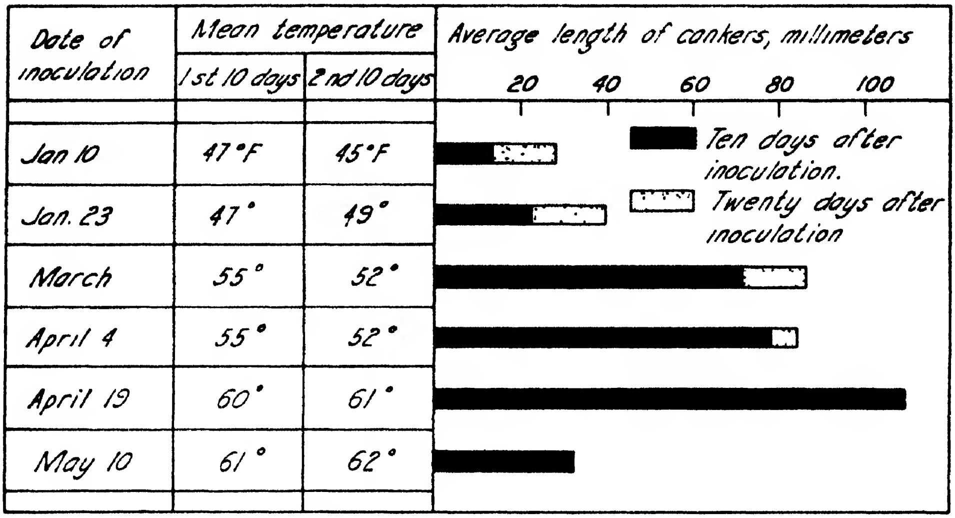

Fig. 3.—Comparative rate of expansion of cankers during the winter and spring of 1934–35. The rate of canker extension for 20 days after inoculation varied; during winter it was more uniform, though not as rapid, as during spring.

which continued to extend. But for inoculations made on April 22 or thereafter, all recorded canker extension occurred in the first 10 days, and none in the second 10.

Discussion of Inoculation Results.—Should the results in figures 1 and 2 and those published earlier (*16,* table 5, p. 116) be plotted, the conformity of the curves would be strikingly similar in major aspects. All the curves are low in early autumn, rise to sudden maxima in late autumn, then decline in midwinter. In early spring again, a second maximum in each curve is apparent; then follows a period when the curves decline to a low point in early summer.

The earlier interpretation of such data tended to discount the importance of the late autumn maximum; but the consistency with which that maximum occurred in three years' results with plum (including those published earlier), and in other results obtained by less systematic inoculations into cherry, have forced the conclusion that it is significant. Since it occurs very near the beginning of autumnal activity in estab-

lished, naturally occurring cankers, it probably results from the same factors that govern renewal of canker activity. In all cases, however, the period during which cankers can extend rapidly in late autumn seems relatively short. Contrasting the duration of rapid canker activity in autumn with that in spring, we find that the latter, although varying

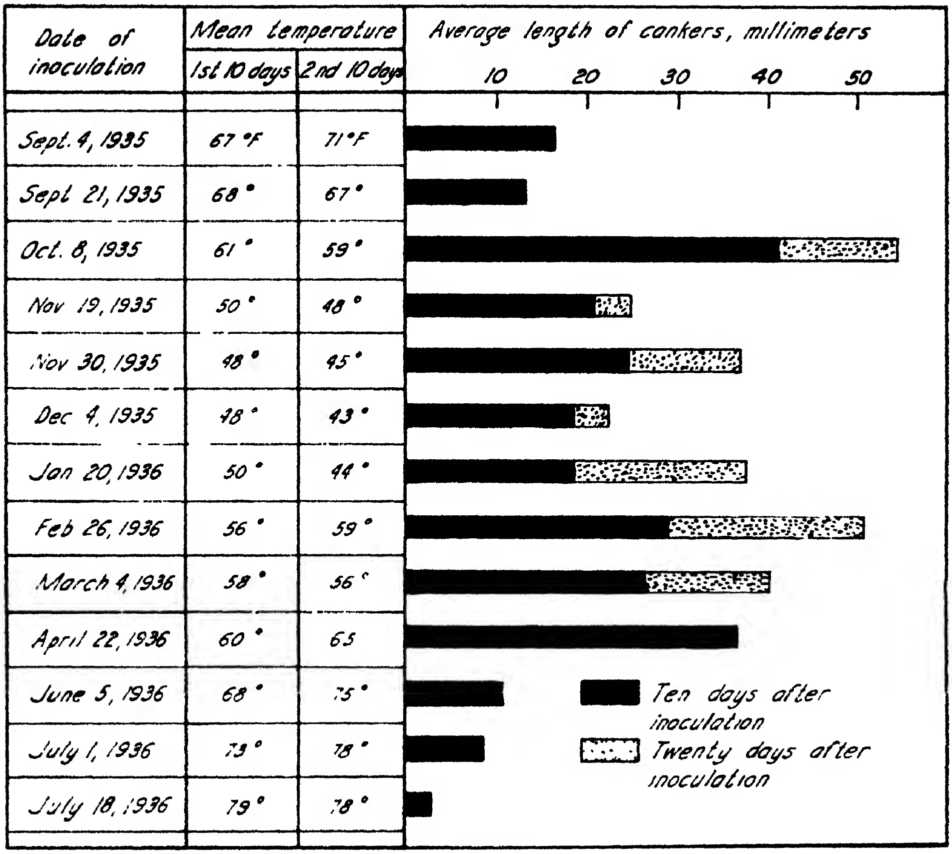

Fig. 4.—Comparative rate of expansion of cankers during autumn, winter, and spring of 1935–36. From late autumn until early spring, the rate of canker extension for 20 days after inoculation appeared to be more uniform than during early autumn or late spring.

from year to year, usually covers a longer period. For an example of this variation, compare the periods of maximum canker development during the springs of 1935 and 1936. The increased activity as spring approaches, as indicated by the experimental results, has its observable counterpart in the greatly accelerated springtime movement of naturally occurring cankers, mentioned in the foregoing section.

At their two extremes we find that curves of the three years' results again coincide closely. This evidence, together with field observations,

proves an almost entire cessation of canker extension during the summer and early autumn months. Hence, activity of the cankers is confined to late autumn, winter, spring, and early summer, and thus exhibit an interesting seasonal aspect. The remainder of this paper will examine factors that might explain the rise and fall of canker activity.

Differences in the comparative rate at which cankers, resulting from inoculations at different times of the year, extended during two 10-day periods after inoculation (figs. 3 and 4) apparently indicate that some factor, or factors, operated to check canker extension even before the springtime maximum of activity was reached (April 19, 1935, and February 26, 1936). Apparently the factor did not exert such a limiting influence between late autumn and early spring as in early autumn and late spring. If the decline in length of cankers resulting from inoculations after the springtime maximum is caused by the same factor, or factors, which before and during the springtime maximum caused a decline in activity, then the factor must have exerted more influence as spring passed and summer approached. In other words, the ability of the factor to limit canker elongation became progressively greater.

RELATION OF TEMPERATURE TO RATE OF CANKER EXTENSION

An examination of the mean temperature prevailing during the 20 days after each series of inoculations noted in figures 1 and 2 will at once suggest that low temperatures during midwinter retarded canker development. A similar relation was found in the earlier work (*16*), where for example a lowering of mean temperature from 70° F after the October 31 inoculations, was accompanied by a correspondingly great decrease in canker extension. Likewise, a mean temperature of 45° after the February 2 inoculations was accompanied by the formation of small lesions; but when the mean temperature following the March 8 inoculations was 57° much larger cankers developed.

As figure 1 of the present paper shows, the autumnal maximum of canker development occurred in inoculations made November 28. The temperature for 20 days after this date averaged 51° F. The next series of inoculations, December 18, developed rather small cankers under average temperatures of 44°.

Although data obtained of canker development during midwinter, 1935–36 (fig. 2) are variable, nevertheless they suggest that canker development was slower during the coldest part of the winter than during the higher temperatures of late fall or early spring.

Results of the following experiment show, apparently, an effect of

temperature on canker extension in winter. On January 10, 1935, and again on January 23, 1935, inoculations were made on the north and south sides of cherry limbs that were growing in an essentially vertical position. Measurements of the January 10 series on February 21 gave for cankers on the south side an average length of 54 millimeters; those on the north side, 30 millimeters. In measurements of the January 23 series on February 27, cankers on the south side of limbs averaged 68 millimeters; those on the north side, 20 millimeters. Although the mean air temperature was 49° F throughout both experiments, the temperature of the surface of the bark was doubtless greater on the south side than on the north side of the limb.

According to earlier experiments (*16*), when potted dormant trees were inoculated and held at controlled temperatures, 65°–70°F was more favorable to canker extension than 50°, and 50° was definitely more favorable than 36°. In a similar experiment in which potted dormant trees were inoculated and held at 36°, 41°, 50°, 60°, and 70°–73°, the last-named temperature was the most favorable for canker extension during the first 3 weeks. Soon thereafter, however, the trees started to grow; and 6 weeks after inoculation, examinations showed the diseased areas to be buried beneath new host tissue. Apparently the cankers had made no further progress after 3 weeks. Trees held at 60°, on the other hand, had started to grow but had not buried the cankers. By the end of 6 weeks, larger cankers were present in these trees than in those held at 70°–73° or at the lower temperatures.

One experiment with potted trees in full leaf was carried out at a controlled temperature of 52° F and also in a greenhouse where the temperature fluctuated between 72° and 75°. As with all the experiments described above, the culture had been tested in orchard trees a few weeks earlier and had proved capable of producing large cankers. To prevent drying out of the inoculations the needle punctures were covered with strips of "Parafilm," a material used to some extent to reduce desiccation of tubed culture media. Upon examination of the trees at the end of 2 weeks, only 3 of the inoculations were found to have produced cankers, and these were less than 10 millimeters long. No effect of temperature was noticeable. In this case, according to the other controlled experiments, the highest temperature (72°–75°) was not above the optimum for canker development, but was within a range more favorable to the disease than the lower temperature (52°); yet no differential effect was noticeable. The trees, in leaf in this experiment, were dormant in the other experiments. Rather than anticipate certain information to be presented later in the paper, the implications of these results will not be discussed here.

To determine whether temperature caused the development of smaller cankers during early autumn and late spring than during late autumn and early spring is more difficult. The 1934 inoculations (fig. 1) made on September 15 and October 20, for example, produced comparatively small lesions. The mean temperatures following these inoculations were 67° and 59° F respectively. In 1935, furthermore, inoculations (fig. 2) on September 4 and September 21, when the mean temperatures were 69° and 67° respectively, also produced comparatively small cankers. These temperatures were all below that (70°–73°) at which rapid initial canker extension occurred in controlled-temperature experiments. Since, however, the maximum temperatures after inoculation were above 70°–73°, we cannot rightfully compare the conditions of the two experiments. Since equipment has not been available for maintaining constant temperatures above 73°, little is known as to the optimum or maximum for canker extension. In an attempt to determine the effect of high temperatures under orchard conditions, Duarte plum, President plum, and Bing cherry were inoculated on May 24, 1937. The mean maximum temperature for the following 8 days was 89°; the mean minimum, 51°. During this time comparatively small cankers were produced in Duarte plum, but much larger ones in President plum and Bing cherry, although not so large as those produced in the same trees earlier in the spring. In such an experiment the unavoidable difficulty is to evaluate the effects of the wide diurnal temperature fluctuation. Possibly, had the temperature remained constantly at 89°, even smaller cankers would have developed in President plum and Bing cherry. One may logically conclude, on the other hand, that high temperature alone did not cause the differences in size of lesions between Duarte plum and the other two species. Some other factor must have been operating to restrict canker extension. Later, evidence is presented to show that at other times of the year canker development differs among the three hosts in the manner just indicated. Apparently, therefore, these differences are caused by conditions inherent in trees of the different types.

According to data in the foregoing section, cankers extended in spring and autumn at different rates during two 10-day periods after inoculation. In some cases the mean temperature varied little during successive 10-day periods. Thus on April 19, 1935 (fig. 3), the mean temperature of the first 10 days after inoculation was 60° F, and canker extension was rapid; but during the second 10 days, when the mean temperature was 61°, no canker development was noticeable. Similar instances are seen in figure 4. Rate of canker extension during late autumn and winter was, on the other hand, more uniform during the two periods. Despite the lack

of data on optimum temperature for disease activity, there is no justification for concluding that temperature wholly explains the unequal extension noted above. More likely, some other factor was operative only during certain times of the year. The trend of the data in figures 3 and 4 suggests that this factor became influential at a time (early spring) when increasing temperature favored increased canker activity. Note, for example, the coincidence between increased rate of canker extension during the first 10 days and the decreased rate of extension during the second 10. In 1935 the springtime maximum of canker activity was reached on April 19 (fig. 3), but the factor had so marked an influence as to prevent canker extension longer than 10 days after inoculation. When on May 10 inoculations were again made, canker extension was a fraction of that in April. Interestingly enough, the mean temperature after the April 19 and May 10 inoculations was the same.

The data, therefore, fail to reveal a direct effect of temperature on the decreased rate of canker elongation apparent (figs. 1 and 2) after springtime maximum; nor is there convincing evidence that the lack of canker development in early autumn was the direct result of unfavorable temperature.

RELATION OF CERTAIN OTHER EXTERNAL FACTORS TO CANKER DEVELOPMENT

Effect of Soil Fertility.—That soil fertility, especially as regards nitrogen, might affect bacterial canker was brought to the writer's attention in the spring of 1934. The disease, having attacked a planting of two-year-old Albright peach trees, was causing noticeably less damage to a number of trees fertilized in the winter with 2 pounds of ammonium sulfate each. In April, as soon as the situation was noted, the remaining unfertilized trees were grouped according to severity of infection. Those already girdled by the cankers, being obviously beyond hope of recovery, were excluded; but those in which the cankers had not girdled the trunk were divided into two classes—moderately diseased and slightly diseased. Half the trees of each class were fertilized with 2 pounds of ammonium sulfate each. A subsequent irrigation insured incorporation of the fertilizer in the soil.

By midsummer the fertilizer had apparently benefited the trees somewhat: new shoots were produced on unaffected parts of limbs below cankers, and limbs attacked but not girdled by cankers sent out new lateral growth. Many unfertilized trees died entirely or lost most of their limbs. The survivors made poor growth. That the fertilizer did not noticeably affect resistance to subsequent infection was apparent the

next winter, when fertilized and unfertilized trees were attacked with equal severity.

In two other instances heavy nitrogen applications stimulated moderately diseased plum trees to a vigorous development of new growth from unaffected portions of limbs below cankers and thereby, apparently, enabled some trees and portions of trees to survive the summer.

Fifteen six-year-old President plum trees were fertilized with 2 pounds of ammonium sulfate each in January, 1935. Rains, shortly after the treatment, incorporated the fertilizer in the soil. Late in February, limbs of fertilized trees and of adjacent unfertilized trees were inoculated with the pathogen. Examinations for the next 4 months failed to show any noticeable effect of the fertilizer on rate of cankers developed, although fertilized trees produced somewhat greater shoot growth and greener foliage than unfertilized trees.

The results are tentatively interpreted as follows: An increased nitrogen supply does not enable the tree to resist infection nor to resist markedly the inroads of cankers during spring. It appears beneficial, however, since trees with abundant nitrogen are better able during the summer to recover from effects of the disease than are trees with less nitrogen. One should remember that at this time when the nitrogen is stimulating growth of the tree, the canker activity is waning or is already at its lowest ebb; hence trees that are weakened but not killed at the time the cankers stop growth might survive the summer if temperature and moisture were favorable. Instead, the advent of high temperature and of conditions favoring high transpiration throws a great strain on the trees; common complications then arising are sunburn and attacks by the fruit-tree bark beetle (*Scolytus rugulosus* Ratz.). Girdled branches either fail to start growth or else put forth small, yellowish leaves and die during the first hot days of summer. If cankers partly encircle the branch, leaf development may also be slow and sparse (fig. 5). Under cool, spring conditions these branches remain alive and, growing slowly, produce new tissue by which to support the portions distal to the canker. For this reason, apparently, the nitrogen, in stimulating the production of new foliage and new lateral shoots, aids the trees to repair in some measure the damage done by the disease.

Beard and Wormald (*1*) found no apparent effect of nitrogen on the size of cankers produced by inoculating with *Phytomonas mors-prunorum* Wor. According to them the largest cankers appeared in trees grown in sand culture and supplied with a "complete nutrient" or a "quadruple phosphate"; the smallest appeared in trees supplied with a nutrient solution lacking in phosphate.

Effect of Soil Moisture.—Field observations furnished conflicting evidence as to the importance of soil moisture to canker development. In orchards where irrigation practices varied greatly, there were no indications that the disease was affected. Some orchards, for example, were not irrigated after the fruit was picked, so that 2 or more months elapsed between the last irrigation and the first winter rains. Other orchards

Fig. 5.—Effect of bacterial canker on foliage development in the spring. *A*, A healthy young peach tree. *B*, A peach tree with most of the primary branches diseased, developing small, sparse foliage, some of which has begun to wilt.

were irrigated one or more times after harvest. Two orchards, on the other hand, that had never been irrigated were little affected by the disease, whereas nearby irrigated orchards were badly injured.

Trees in poorly drained areas sometimes appear to suffer worse than those in well-drained areas. In two orchards under observation since 1929, the disease was at first causing greater damage to the trees in poorly drained areas than to those on well-drained slopes, but later it spread over the entire orchard. In these cases, clearly, infection happened first to become established in the poorly drained areas, but thereafter spread to areas where drainage was good.

Experiments were outlined to study canker development in trees given widely different soil-moisture treatments. Before the experiments are described, however, certain terms used in soil-moisture studies must be defined. Investigators have established two reference points (*13*, *14*) with regard to the amount of water in the soil. The first is field capacity, or the capacity of a soil to retain water against gravity. The numerical expression of this value is percentage of water calculated on the basis of oven-dry weight. The second reference point is permanent wilting per-

centage, or that percentage of moisture in the soil (calculated on an oven-dry basis) below which plants cannot readily obtain water and, in consequence, will permanently wilt unless water is supplied.

Plants can obtain water when the moisture content of the soil is between the field capacity and the permanent wilting percentage. Between these two limits, therefore, is the range of "readily available moisture." There seems to be a widespread belief that somewhere within this range lies a moisture content at which plants grow best. Experiments of Veihmeyer and Hendrickson at this station (*4, 5, 14*) do not substantiate this belief. In fact, these workers find that growth of orchard trees and quality of fruit are not affected by differences in the amounts of readily available moisture, but are affected if the soil moisture goes below the permanent wilting percentage early in the growing season.

These investigators (*13, 14*) reveal, moreover, the practical impossibility of maintaining a definite and uniform soil-moisture content within the range between field capacity and permanent wilting percentage. Their papers (*13, 14*) give additional discussion of the principles underlying soil moisture.

Confronting such difficulties, the present writer did not attempt to perform experiments in which a predetermined amount of water added to the soil was assumed to establish a definite and uniform soil-moisture content. Instead the plan was to study the effects of soil moisture on the development of cankers (1) when trees were supplied with readily available moisture at all times and (2) when trees were not so supplied but were growing in soil near or below the permanent wilting percentage. These experiments were conducted on trees growing in the orchard, and on trees growing in tanks[5] holding about a ton of soil.

Orchard experiments were conducted for three years. In 1934 two-year-old Duarte and President plum trees were divided into two plots separated from each other by a guard row. In late summer one block was left unirrigated; the other was irrigated at intervals. By early autumn, soil samples taken 3 feet from the base of the trees and to a depth of 6 feet showed that the trees in the unirrigated plot had reduced the soil moisture to a low level.[6] The irrigated plot was then given a heavy irrigation to a depth of at least 3 feet. The trees in both plots were then inoculated with a water suspension of the pathogen.

The 1934 results, together with the soil-moisture determinations which appear in table 2, show that only small lesions were developed by the

[5] The writer wishes to thank Dr. A. H. Hendrickson and Dr. F. J. Veihmeyer for their aid in planning the experiments and in lending the tanks and equipment.

[6] The permanent wilting percentage of the top 4 feet of this soil has been determined by Hendrickson and Veihmeyer to be between 11 and 12 per cent.

inoculations, those on the irrigated trees being slightly smaller than those on the unirrigated. A further difference is noted in the number of inoculations producing cankers: a larger percentage of those on unirrigated trees developed symptoms than did those on irrigated trees. The small differences here reflected are readily explained as follows: Irrigated trees, retaining the ability to produce new tissue around the inoculation wound, promptly buried many small lesions beneath xylem.

TABLE 2

CANKER DEVELOPMENT IN IRRIGATED AND UNIRRIGATED ORCHARD TREES

Date* experiment was started	Variety of tree used	Unirrigated trees					Irrigated trees				
		Per cent of inoculations producing cankers	Average length of cankers, millimeters	Per cent soil moisture at depths stated†			Per cent of inoculations producing cankers	Average length of cankers, millimeters	Per cent soil moisture at depths stated†		
				0–2 feet	2–4 feet	4–6 feet			0–2 feet	2–4 feet	4–6 feet
Sept. 1934	Duarte plum	61	15	11.5	15 2	14 7	48	10	22 4	22.0	17.5
Oct. 1934	President plum	90	12	11 3	14 4	12 5	56	7	20 9	21 9	14.1
Oct. 1935	President plum	82	13	10 9	13 9	12 5	61	9	25 7	25.5	20 6
Nov. 1936	Duarte plum	84	25	10 8	11 8	11 4	90	20	24 0	22 2	18.6
Nov. 1936	Bing cherry	92	35	10 8	11 8	11 4	87	35	24.0	22 2	18 6

* The irrigation and inoculation of trees were made in these months; the cankers were measured 23 to 26 days after inoculation

† Per cent of moisture based on oven-dry weight of soil.

Since the unirrigated trees, on the other hand, did not possess this ability, a higher percentage of the inoculations produced small cankers. These results, however, reveal no marked tendency for cankers in trees of one treatment to develop further than those in trees of the other treatment. It should be mentioned that, though the moisture in the top 2 feet of soil was at the permanent wilting percentage, the moisture of the soil from 2 to 6 feet in depth was somewhat above that percentage. Hence the trees in the unirrigated plots had a small amount of available moisture at the beginning of the experiment.

Repetitions of this experiment in 1935 and 1936 (table 2) gave no convincing evidence that differences in soil moisture were affecting canker development. The 1936 inoculations produced, in the same length of time, larger cankers than did those of 1934, presumably because they were made later in the autumn. In this year no difference whatever was found between the length of cankers in either the plum or the cherry

trees receiving the two treatments, though apparently soil moisture to a depth of 6 feet was at the permanent wilting percentage.

In the winter of 1934–35 young Duarte plum trees were planted in tanks holding about a ton of soil. The field capacity and the permanent wilting percentage of this soil had previously been determined. Since the tanks were equipped for weighing, the trees could be given identical treatment with regard to water. So that the roots of the trees might penetrate all the soil in the tanks, the differential moisture treatments were not begun until early autumn, 1936. In late summer of that year half of the tanks were left unwatered. The other half were maintained considerably above the permanent wilting percentage at all times. By mid-October, weighings indicated that the unwatered trees had reduced the soil moisture to the permanent wilting percentage. Additional evidence that this point had been reached was furnished by the trees themselves, in that the leaves wilted and fell. The soil in the irrigated tanks was again brought to its field capacity and was kept wet for the duration of the experiment. Six inoculations were made into each tree on three different dates. The first series was made October 15, the second October 24, and the third October 31. On October 24 four trees that had been allowed to wilt were irrigated when they were inoculated.

TABLE 3

Canker Development in Irrigated and Unirrigated Trees Growing in Tanks, 1936–1937

Treatment of trees*	Date of inoculation	Average length,† in millimeters, of cankers produced by six inoculations in each tree									Average
		1‡	2	3	4	5	6	7	8	9	
Irrigated	Oct. 15, 1936	14	14	14	18	10	11	18	16	18	15
Unirrigated	Oct. 15, 1936	0	1	1	2	5	7	10	0	1	3
Irrigated	Oct. 24, 1936	34	20	30	39	27					30
Unirrigated	Oct. 24, 1936	0	1	0	3	4					2
Unirrigated-irrigated	Oct. 24, 1936	15	7	10	10						13
Irrigated	Oct. 31, 1936	21	19	24	9	20					19
Unirrigated	Oct. 31, 1936	3	4	8	1	2					4
Irrigated	June 5, 1937	33	24	21	29	37					29
Unirrigated	June 5, 1937	4	0	0	2	1					1

* Irrigated—soil moisture kept above the wilting point at all times
Unirrigated—soil moisture brought to the wilting point and kept there during the experiment
Unirrigated-irrigated—soil moisture at the wilting point until inoculations were made, then brought to field capacity for the duration of the experiment

† Measurements made 10 days after inoculation

‡ Numbers 1 to 9 designate the different trees that were inoculated

As shown by the results in table 3, trees of the two treatments differed strikingly in canker development. According to measurements 10 days after inoculations, whereas the bacteria were producing fairly large

cankers in irrigated trees, there was little evidence of canker development in the wilted trees. In June, 1937, this experiment was repeated on 10 of these trees with similar results. When trees previously wilted were irrigated at the time of inoculation, the bacteria proceeded to produce distinct cankers—less extensive, however, than those in continuously irrigated trees.

At first examination it seems impossible to reconcile the divergent results of the two types of experiments. Since, however, results of the tank experiments were so definite, the divergence must have been caused by the dissimilar conditions under which the experiments were conducted. As mentioned earlier regarding the 1934 orchard experiments, trees in the dry plot apparently had a small amount of available moisture at the beginning of the experiment. The same is true of the 1935 experiments. In 1936, on the other hand, the soil in the dry plots was at the permanent wilting percentage to a depth of 6 feet. Since, however, this condition was not reached until late autumn, when trees in both wet and dry plots were losing their leaves, no actual wilting of foliage was observed. Possibly, though most of the roots of these trees were in soil where moisture was at the permanent wilting percentage, a few might have been in moist soil below the 6-foot depth. In this connection Conrad and Veihmeyer (*3*) conclude from their work with sorghum that "if a portion of the root system is partly in dry soil and partly in wet soil, the needs of the plants might be adequately met by adsorption from moist soil."

Apparently, therefore, under conditions in orchard experiments, where most of the roots were in dry soil but where a few roots might have been supplying the trees with moisture, no effect on canker development was exerted. As mentioned earlier, the small differences indicated in the 1934 results can be explained as arising through differences between the ability of the trees in wet and dry plots to bury diseased areas beneath new host tissue. The differential of soil moisture in the field experiments did not appear to make trees strikingly more or less susceptible.

So much for differences occurring in the orchard tests only. The differential in soil moisture obtained in the tank experiments was unquestionably greater. Not only was this found by weighing the tanks, but the trees themselves gave evidence, in that leaves of trees in wet tanks remained turgid, whereas those of trees in the dry tanks wilted and dropped. Unquestionably, therefore, the soil moisture in the dry tanks was below the permanent wilting percentage. The consistency with which cankers failed to develop in the dry trees appears to be adequate proof that lack of readily available soil moisture affected the disease under the extreme conditions obtaining.

Whether the extreme conditions of the tank experiments would occur under orchard conditions, where the tree roots are not restricted to a limited mass of soil, depends upon the depth of the soil, the range of readily available moisture, the characteristics of the soil, and the proximity of a water table to the surface. Trees in the Sierra Nevada foothills, where the soil is shallow and where the range of readily available moisture is narrow, are more liable to suffer extreme drought conditions in absence of irrigation than trees growing in the Sacramento Valley. The unirrigated orchards mentioned at the beginning of this discussion were located in the foothill district and exhibited definite drought symptoms during late summer. The leaves dropped early, and the trees were obviously stunted. This might have been one reason why the disease damaged unirrigated trees very little but caused severe damage in nearby irrigated orchards.

Just how drought effect, coming in later summer when canker activity is at a low ebb, could influence the severity of the disease, is not known. The most likely influence would seem to be on survival of bacteria in the cankers, which would in turn affect the supply of inoculum the following winter. Though this problem was not extensively studied, observations in one winter indicated an apparent difference between an irrigated and an unirrigated orchard in the percentage of active cankers. In the unirrigated orchard, 28 per cent of the cankers were active; in an adjacent irrigated orchard, 71 per cent. Obviously these results are too meager to permit conclusions.

RELATION OF VARIETY OF PLUM TO DISEASE DEVELOPMENT

The behavior of different varieties of plums towards the disease appeared to deserve further study. Since earlier work (*16*) had revealed a marked variability in the reaction of several commonly grown varieties to the presence of cankers in the limbs, it was thought that by observing a few of these varieties, some apparently resistant and others susceptible, one might learn whether or not any common characteristic of behavior existed. Before these studies are discussed, information regarding resistance and susceptibility, other than that already published (*16*), must be given.

Whatever the basis for classifying varieties according to resistance and susceptibility, whether it be the number of trees partially or wholly killed, or the frequency with which the trees are attacked, the result is a composite, the integral parts of which exist for different reasons. If, for example, the basis is tree mortality, one variety may suffer little loss,

perhaps because the trees are inherently resistant to attack or because they are, when attacked, not favorable to rapid extension of canker, or because they somehow escape infection. If, on the other hand, the basis is the frequency with which the disease occurs in trees of the different varieties and if no attention is paid to the number of trees wholly or partially killed, the results will reflect any existing inherent resistance to attack and any escape; but they will not necessarily show how favorable or unfavorable the trees of particular varieties are to extension of cankers once infection is established.

These points are stressed because one can interpret the following data best by keeping in mind the factors contributing to those phenomena called susceptibility and resistance. No claims are made that the data to be given reflect, or could have been made to reflect, the separate influences of the contributory factors. In some outstanding examples to be cited, the severity of the disease in a given variety in a given year was almost certainly conditioned by one or more of these factors.

Susceptibility from the Standpoint of Frequency of Infection.—That differences in incidence of infection may in some years be among the important causes of heavier losses in one variety than in other equally susceptible varieties was recently observed. In 1935 Duarte trees in certain orchards were wholly or partially killed in greater numbers than adjacently growing President, Grand Duke, or Tragedy, mainly because they became infected through blossom buds and apparently, in some cases, through the open blossom itself or through the bases of the blossoms. In 1936 the reverse was true; President trees suffered more from blossom bud infection than did Duarte. In 1937 Duarte, Wickson, and Santa Rosa trees were, on the whole, attacked through buds far more frequently than President, Grand Duke, or Tragedy. From the standpoint of the rapidity of canker extension, once infection is established, President seems somewhat more susceptible than Duarte.

Susceptibility of Different Varieties from the Standpoint of Tree Mortality.—The 1933 classification of the susceptibility of plum varieties was based on records of the percentage of trees wholly or partially killed during the 1930 outbreak. Additional observations considerably alter this list, particularly on varieties falling between the two extremes of susceptibility. Climax, for example, listed as more susceptible than Grand Duke and Tragedy, seemed in later years considerably less affected than they. One should not infer, however, that in all years the three varieties differ widely with respect to the number of trees lost. One reason for the seeming change from one year to another, of comparative susceptibility between different varieties was given under the preceding heading.

A further change in order of susceptibility concerns the Sugar variety, which is undoubtedly more susceptible than indicated by its position in the list (*16*).

Regarding the most susceptible and most resistant varieties the list needs little revision: President and Duarte have consistently suffered greater loss than any others; Kelsey and Beauty, very little loss.

Table 4 shows the wide difference of susceptibility between a few important varieties. To minimize differences arising from the existence of

TABLE 4

Susceptibility of Different Varieties of Plums to Bacterial Canker

Year observations were made	Orchard number	Variety of plum*	Per cent of trees badly diseased†
1933	1	President	41
		Santa Rosa	16
		Beauty	12
1933	2	Grand Duke	70
		Sugar	58
		Kelsey	5
		Burbank	12
1934	3	Grand Duke	25
		Beauty	11
1937	4	President	27
		Duarte	13
		Beauty	0

* President, Grand Duke, and Sugar are *Prunus domestica* varieties, Santa Rosa, Beauty, Kelsey, and Burbank, *P. salicina* varieties; Duarte appears to be a *P. salicina* hybrid

† This includes trees in which the disease was severe enough to kill branches or the entire tree

localized infection centers that might have become established in one block of trees and not in another, observations in a given orchard were confined to adjacently growing trees of the same age. These data show the consistently high susceptibility of President, Grand Duke, and Sugar in contrast to the resistance of such varieties as Beauty and Kelsey. Burbank and Santa Rosa appear intermediate. Duarte trees (observations of 1937) were affected much more than Beauty but not so badly as President when the Duarte trees were adjacent to the other two varieties. This difference should be remembered during later discussion.

Differences in the Rate of Canker Extension in Varieties of Plums.—The comparative susceptibility of President and Duarte plum has been approached from the standpoint of the rate at which cankers extend in the limbs of each. At various times during 1935, 1936, and 1937 adjacently growing trees of the two varieties were inoculated. The trees were

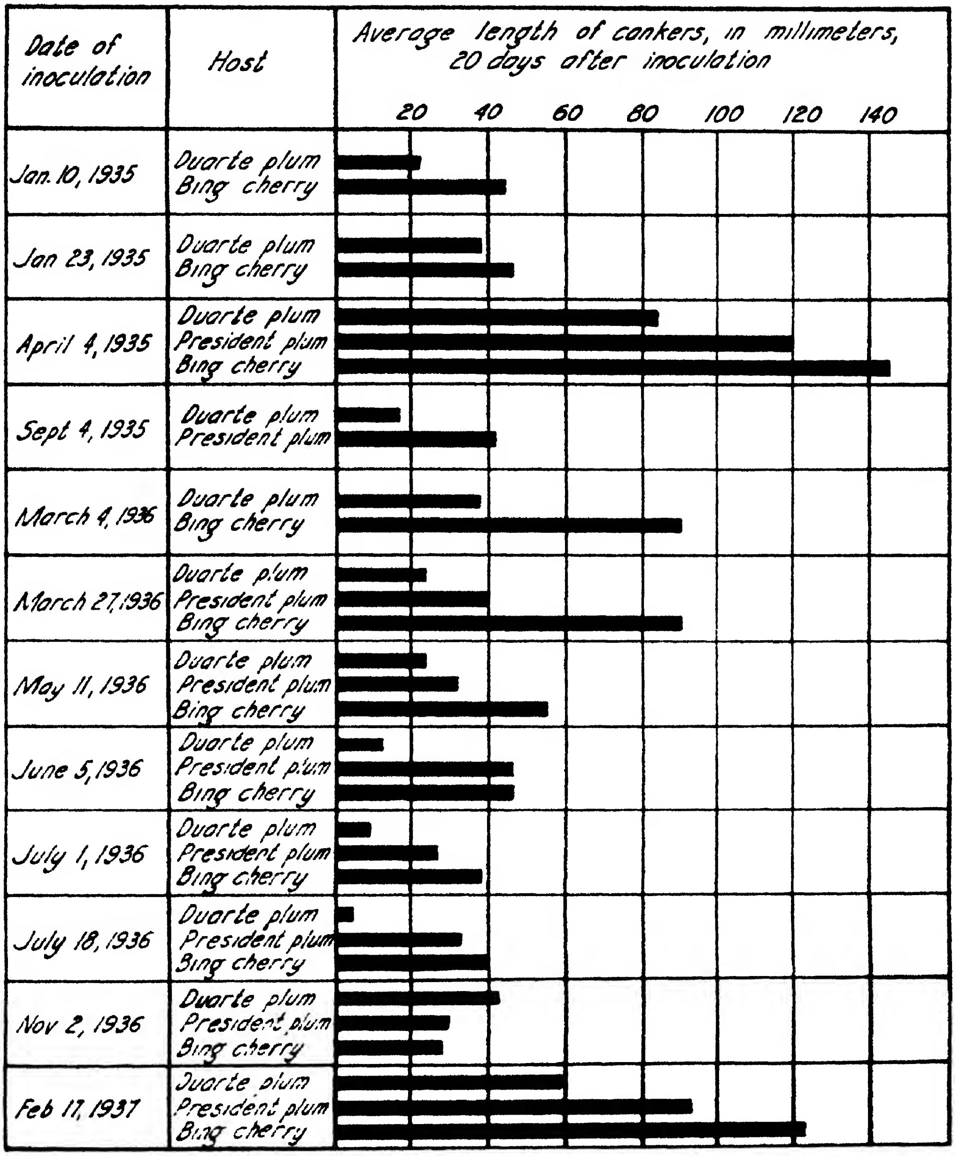

Fig. 6.—Canker development in two varieties of plums and one variety of sweet cherry. The data indicate that Duarte plum trees are somewhat less favorable to extension of cankers than President plum and Bing cherry trees.

of the same age, having been planted in the winter of 1931–32. For comparison Bing cherry trees of the same age were also inoculated. Cankers were measured 20 days after inoculation.

According to figure 6 larger cankers were produced in limbs of the variety President than in Duarte, with but one exception. As might be

expected from the inoculation results given earlier in this paper, cankers produced at different times of the year varied in size. This was true of both plums and the cherry as well. With but two exceptions, cankers produced in the cherry limbs were somewhat larger than those produced in President.

Here we see further evidence for regarding Duarte as less susceptible than President, even though in some years more Duarte than President trees were wholly or partially killed. This work raises the question as to whether or not other varieties of plums differ with respect to the comparative rate of canker extension after infection occurs. Though no comparable body of data is available on the point, one series of inoculations made January 12, 1938, in Placer County leads one to believe that other varieties do differ in respect to the rate of canker development. The average length of canker, 44 days after inoculation, for several varieties of plums is tabulated as follows:

Variety	Millimeters
President	89
Grand Duke	64
Sugar	57
Duarte	45
Burbank	45
Beauty	19
Kelsey	12

According to these data, the European type of plum (President, Grand Duke, and Sugar) developed larger cankers than the Japanese type (Duarte, Burbank, Beauty, and Kelsey). In the previous section, the Beauty, Kelsey, and Burbank varieties were said to suffer less loss of trees than such varieties as President, Grand Duke, and Sugar. The inoculation results indicate a possible reason for the difference in susceptibility, in that cankers in Kelsey and Beauty developed less rapidly than those in President, Grand Duke, and Sugar.

INTERNAL REACTIONS OF THE HOST IN RELATION TO CANKER DEVELOPMENT

We shall now consider the morphological changes in the host tissue that might be construed as reactions to the bacteria and shall attempt to determine whether such changes affect canker activity.

Nature of the Reactions.—Strictly speaking, reactions to the bacteria are of two types: (1) those in which the vascular cambium, or at least undifferentiated cells in that region, are concerned; and (2) those in which a cork cambium or phellogen is concerned. Both these types of reactions may occur in different parts of the same canker.

Frequently the margins of cankers advance only along a restricted zone in the vascular cambium. The bacteria may later invade the overlying phloem and cortical tissue; but often diseased areas in the cambial region at the margins of the cankers will be buried beneath new tissue generated by unaffected cambial cells—or at least by undifferentiated cells in the cambial region—before the bacteria have time to invade the phloem and cortex. This new tissue, interestingly enough, is xylem laid down in a more or less regular fashion. As shown in figure 7, the new developing

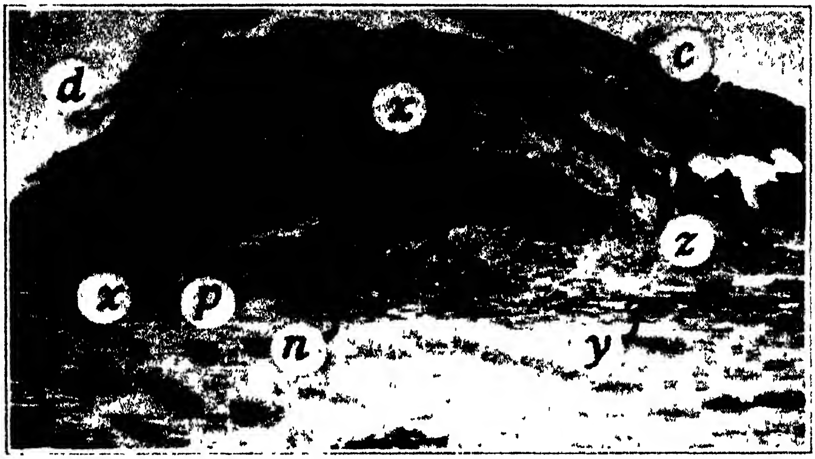

Fig. 7.—Radial longitudinal section through the bark of a Duarte plum branch inoculated on September 15, 1935, fixed two months later, showing how part of the diseased phloem tissue (p) is buried by later-formed xylem (x). Infected phloem tissue (d) has been separated by means of a phellogen from remaining healthy phloem and cortex (c). At y the disease has extended along what were then outer layers of sapwood, buried by new xylem (x) through activity of the cambium (z). Apparently the only derivative of this cambium is xylem. The path of the needle is shown at n and in the immediate neighborhood some of the tissue has become infected; this diseased tissue extends along y.

xylem may, in needle-prick inoculations, occlude some phloem tissue at the point of inoculation. Since the host responds similarly, but less extensively, near wounds made with a sterile needle, we cannot attribute the response evident around the needle wound in figure 7 as stimulated wholly by bacteria. The bacteria have, however, invaded (d) above and below the path of the needle (n). When the invasion occurred the lowermost area of diseased tissue was in the cambial region but was subsequently buried beneath new xylem (x). This type of host reaction departs but little from the normal production of new tissue by the cambium. The bacteria had invaded considerable phloem (d), but when the fixation was made this infected area was separated from the overlying healthy

phloem and cortex (*c*) by a periderm. The response involved the production of a phellogen, which will be considered next.

A phellogen develops at the lateral margins of a canker and generates a mass of new tissue similar in some respects to that developing at wounds. If the bacteria have invaded the bark tissue downward to the cambium both at the apices and the lateral margins of the canker, the mass of new tissue may extend entirely around the infected area. More frequently, however, such tissues are formed only along the lateral margins; the apical margins will be seen to diffuse into a series of loosely connected reddish-brown streaks, forming a zone sometimes several inches wide. Microscopic examination of cross sections of these reddish-brown dormant streaks will show a core of infected tissue surrounded by a periderm sometimes 10 to 15 cells thick. The cross-section area of the streaks varies from only a few cells to many hundred, the number depending on the amount of tissue invaded when the periderm was formed. The thickness of the periderm varies among different diseased areas. Extremely vigorous periderm formation sometimes occurs when the cankers are located in the outer layers of the bark; the inner face of the canker is separated from the underlying healthy tissue by a phellogen that generates a periderm many cells thick. When exposed the periderm cells may separate from each other and fall apart as a powdery mass; and by breaking the outer layer of bark one may lift the entire diseased, dead area away from the underlying healthy tissue. A somewhat similar situation exists in the pear-blast canker caused by the same organism (*17*, *18*).

Although not studied in detail, the major anatomical changes occurring when phellogen appears around infected streaks at the apices of cankers have been noted. The first noticeable sign of phellogen formation was an apparent clearing of a zone of healthy cells at the periphery of infected streaks. The cause of this clearing, noticeable in freehand sections of fresh material, was not determined. Priestley (*8*), noticing a similar clear zone, attributed it to the absence of air between the cells. A translucent, watery appearance of cells near the vascular cambium just before cambial activity in the spring was said by Priestley (*8*) and by Priestley and Swingle (*9*) probably to mean that the cell contents had changed from a condition of a gel to one of a sol. In the present work the clearing first occurred in cells on the face of the diseased area nearest the vascular cambium. Shortly thereafter certain cells in the cleared zone laid down walls parallel to the margin of the diseased area. New cells were then generated by this layer. Figure 8 shows three stages: (*A*) A cross section through diseased bark before the phellogen appears. Since, in this case, the bacteria did not involve all the cells near the invasion,

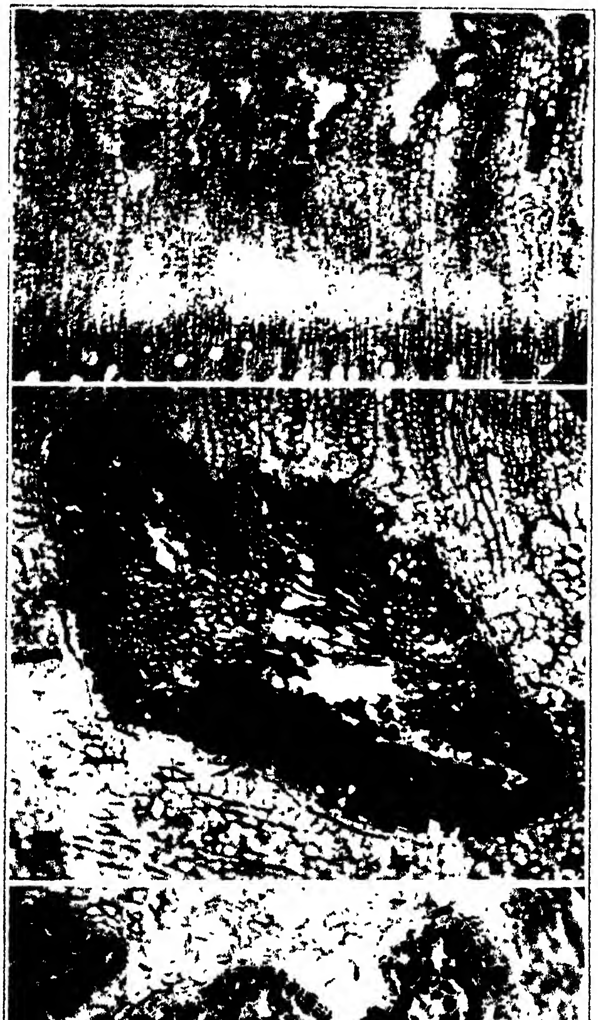

Fig. 8.—Cross sections through diseased bark of plum in different stages of phellogen development. *A*, Before phellogen has formed; note the indefinite, diffused region of invaded tissue. *B*, Well-defined, gum-impregnated invaded region after a phellogen has formed. *C*, A well-developed periderm separating infected from healthy tissue.

the limits of the diseased area are ill-defined. (*B*) A cross section through a diseased area around which phellogen has formed and has begun to produce new cells. The diseased region is now well defined, and most of the cells in the region are involved. (*C*) A cross section through a diseased region surrounded by periderm 6 to 7 cells thick.

Relation of Host Activity to Periderm Formation.—From the work of others (*8*) we know that cambial activity, initiated at the base of the bud when the buds begin to grow, spreads to all parts of the tree. Bud growth

TABLE 5

DEVELOPMENT OF PERIDERM AROUND INVADED AREAS IN THE BARK OF AN EARLY AND A LATE BLOOMING VARIETY OF PLUM, 1935

Date of fixation	Duarte (early blooming)		President (late blooming)	
	Stage of host development	Occurrence of phellogen	Stage of host development	Occurrence of phellogen
Feb. 21	Closed cluster	None	Dormant	None
Feb. 27	Open cluster, tips of two leaves exposed	None	Buds swelling	None
Mar. 12	Full bloom, two leaves unfolded	Beginning	Closed cluster	None
April 4	Ten leaves unfolded, largest 53×18 mm	Well developed	Just past full bloom, three leaves unfolded, largest 20 ×10 mm	Beginning

is, therefore, a visible criterion of cambial activity; those varieties of plums that blossom earliest will start cambial activity earliest. As far as the reaction of the tree to the presence of *Phytomonas cerasi*, as just outlined, concerns the activity of vascular cambium at the margins of invaded and healthy tissue, those varieties which bloom earliest will presumably be the earliest to produce new tissue in such areas. But, since reaction of the host to the presence of the bacteria also involves periderm formation in phloem and cortical tissue, it was necessary to study the relation between this phenomenon and growth of the tree.

To define the connection between the beginning of growth of the host in the spring and periderm formation around diseased areas in phloem and cortex, the following studies were made. Limbs of Duarte plum trees were inoculated in early February, 1935. At 3- to 4-day intervals, pieces of tissue at the apices of the developing cankers were examined for phellogen. As long as the trees remained dormant no phellogen was produced, but when the buds started growth a phellogen appeared and began to generate periderm. In these particular cases about three weeks elapsed

between inoculation and the first observed phellogen. The time necessary for phellogen development after inoculations in late May was, on the other hand, between 9 and 11 days.

Thus it seems that periderm formation around invaded areas in phloem and cortex appears also to be determined by activity of the tree. Between late autumn and early spring or, in other words, during dormancy of the trees, it was not observable.

Periderm Formation around Diseased Areas in Different Varieties of Plums.—Since periderm formation around invaded areas begins in the

TABLE 6

RELATION OF VARIETIES OF PLUMS TO CERTAIN CHARACTERISTICS OF THE CANKERS DURING THE SUMMER OF 1932

Variety*	Species	Per cent of cankers with reddish-brown streaks at margins of necrotic area
Clyman	*Prunus domestica*	15
Sugar	*P. domestica*	5
Grand Duke	*P. domestica*	18
Tragedy	*P. domestica*	28
California Blue	*P. domestica*	26
Duarte	*P. salicina*†	58
Santa Rosa	*P. salicina*	73
Wickson	*P. salicina*	78

* Santa Rosa, Duarte, and Wickson varieties blossom much earlier in the spring than do the other varieties.

† Duarte appears to be a *Prunus salicina* hybrid.

spring only after the trees start growth, it should differ in varieties that started growth at different times. Two varieties of plums, one of which (Duarte) begins growth 2 to 3 weeks earlier than the other (President), were inoculated February 10, 1935. Freehand sections of the cankers at different times after inoculation showed that the Duarte began to form a periderm around infected tissues about 3 weeks before the President (table 5). The first observed periderm appeared in both varieties at about the same stage of their development—that is, just before full bloom.

No similar body of data is at hand regarding development of a periderm in other varieties. Such studies would be valuable, particularly in the varieties Beauty and Kelsey, which besides starting growth early are much more resistant to the disease than Duarte. Judging from some observational data, however, Kelsey and Beauty tend to produce periderm earlier in the spring than such varieties as President, Grand Duke, and Tragedy. Early in the work the characteristics of cankers during the

summer were found to differ among varieties. At first no particular importance was attributed to this fact, but later evidence showed that these differences were caused by the presence or absence, and by the relative amount, of periderm around infected streaks at the apices of cankers. Cankers, for example, could be separated on the following bases: (1) those in which the dull-brown, watery margins gradually merged into

TABLE 7

COMPARISON OF THE VARIETIES OF PLUMS WITH RESPECT TO CERTAIN CHARACTERISTICS OF THE CANKERS DURING MAY, 1935, AND MAY, 1937

Date of observation	Variety*	Species	Class 1†	Class 2‡	Class 3¶
May 29, 1935	President	*Prunus domestica*	58	26	16
	Grand Duke	*P. domestica*	32	33	35
	Tragedy	*P. domestica*	40	40	20
	Duarte	*P. salicina*	18	38	44
	Beauty	*P. salicina*	6	17	77
	Santa Rosa	*P. salicina*	0	1	99
May 19, 1937	President	*P. domestica*	66	34	0
	Grand Duke	*P. domestica*	53	47	0
	Tragedy	*P. domestica*	31	54	15
	Duarte	*P. salicina*	6	56	38
	Beauty	*P. salicina*	0	39	61

* The order in which these varieties blossom in the spring is: Santa Rosa, Beauty, Duarte, Tragedy, Grand Duke, President—the Santa Rosa being earliest and the President latest.

† Class 1, cankers with few or no dormant streaks.

‡ Class 2, cankers with a considerable number of dormant streaks.

¶ Class 3, cankers with most of the visibly invaded tissue in the form of dormant streaks.

healthy tissue, with no reddish-brown dormant streaks, and (2) those in which the margins were well defined at the edges of the necrosed area and which also possessed a zone of the reddish-brown dormant streaks beyond the necrosed area. Data in table 6 collected during the summer of 1932 reveal a striking difference between varieties in the percentage of cankers belonging to the second type. Trees of *Prunus domestica* varieties bore markedly fewer cankers of this type than did those of P. *salicina* varieties.

In May, 1935, and May, 1937, further evidence on this point was obtained. Based upon the character of the canker with respect to the presence or absence and the relative abundance of dormant streaks, three classes of cankers were obtained: (1) cankers having very few or no dormant streaks at the apices, (2) cankers having a considerable number of dormant streaks intermingling with the dull-brown, active streaks, and (3) cankers having most or all of the visibly infected tissue at the apices in the form of the reddish-brown dormant streaks. Only such cankers

as had enlarged during the previous winter and spring were included. Those that had girdled the limb were omitted, since presumably such weakened limbs would not react to the presence of the disease with the same vigor as ungirdled limbs. According to data presented in table 7 the *Prunus domestica* varieties again separated themselves from the *P. salicina* with regard to the abundance of infected streaks surrounded by periderm. In this respect Duarte stood intermediate between President on the one hand and Beauty on the other. Apparently, therefore, the early varieties are first to start periderm formation.

Callus Formation at the Surface of Mechanical Wounds in Different Varieties of Plums.—Response of different varieties of plums to wounds inflicted during dormancy was observed for two seasons. Although the response to surface wounds might differ in certain details from that occurring at the margins of diseased areas in the interior of the bark, the presumption was that the wound response, in regard to its vigor and to the time it began in the spring, would coincide with the response at the margins of invaded areas.

Trees of about the same age and growing in nearby plantings given the same cultural treatments were used in these studies. Early in February 1-inch holes were bored into 5 limbs of each of 5 trees of the different varieties. The holes, extending through the cambial region to the sap wood, were painted with shellac in accordance with the work of Marshall (*6*), who found that shellacking aided callusing in forest and shade trees. At different times thereafter the wounds were observed for callus, which first developed as a light-green, cheesy mass of tissue at the lateral sides of the holes. The length and width (at the widest point) of these callus masses were measured at two different times. The measurements of length were multiplied by the measurements of the width to obtain a callusing index. These indices, together with information concerning stage of development of the different varieties at the time the callus was measured, appear in table 8.

In 1934 the experiment included three varieties: President, one of the latest plums to start growth; Beauty, moderately early; and Santa Rosa, very early. By March 9, according to the data in table 8, Beauty and Santa Rosa, but not President, had developed a measurable amount of callus. By March 22, President had developed considerable callus, but the other two varieties were far ahead, Santa Rosa more so than Beauty.

The 1935 experiments included seven varieties that differed both in their susceptibility to the disease and in their earliness to start growth. In addition younger trees (nine years old) of two of the varieties were studied. Callusing began when the blossom buds were opening (table 8).

The first to begin were the earliest-blooming varieties, Santa Rosa being the earliest. There was a great difference between *Prunus domestica* varieties (President, Grand Duke, and Tragedy) and those of *P. salicina* (Kelsey, Beauty, Santa Rosa, and Duarte); the latter group began to form callus earlier than the former. In addition the young Beauty trees were somewhat later to bloom and later to begin callusing than the older Beauty. This, however, was not the case with young President.

TABLE 8

CALLUSING OF WOUNDS IN DIFFERENT VARIETIES OF PLUMS

Year	Variety	First observation*			Second observation		
		Host development†	Per cent of holes callusing	Callusing index‡	Host development†	Per cent of holes callusing	Callusing index‡
1934	Santa Rosa	End of bloom	80	16	Fruit 4–5 mm diameter	100	99
	Beauty	Full bloom	64	10	Fruit 3–4 mm diameter	96	40
	President	Dormant	0	0	10 per cent blossoms open	60	13
1935	Santa Rosa	Open cluster	54	5	5 leaves unfolded	100	38
	Kelsey	Early open cluster	44	4	7 leaves unfolded	96	24
	Duarte	Early open cluster	44	4	4 leaves unfolded	100	32
	Beauty	Early open cluster	60	6	1 leaf unfolded	93	23
	Beauty (young trees)	Buds swelling	28	3	Full bloom	68	8
	Tragedy	Buds swelling	0	0	50 per cent blossoms open	74	9
	Grand Duke	Dormant	0	0	15 per cent blossoms open	48	4
	President	Dormant	0	0	Open cluster	40	2
	President (young trees)	Dormant	0	0	Open cluster	70	3

* First observation in 1934 was March 9, and in 1935, February 18; second observation in 1934 was March 22, and in 1935, March 19.

† Although most designations of the stages of host development are self explanatory, the following are defined: "buds swelling" and "buds breaking" refer to the enlargement and separation of the scales of the buds containing blossoms; "early open cluster" and "open cluster" indicate different stages in the separation of individual blossoms in clusters after they have emerged from the bud scales.

‡ Index number derived by multiplying length of callus tissue by its width.

Relation of Host Development and Periderm Formation to Canker Development.—Three different methods of approach indicated in the foregoing section that those varieties first to start growth in spring were also first to produce periderm around diseased areas or callus at the surface of wounds. If we now compare these data (tables 5, 6, 7) with those (table 4) concerning susceptibility of varieties we find, on the whole, that the varieties first to start growth (Kelsey, Beauty, and Santa Rosa) were among the more resistant, while those starting growth later (President,

Grand Duke, and Tragedy) were the more susceptible. An apparent exception was Duarte, which, though highly susceptible to infection through buds, appeared less favorable to extension of cankers than President. In other words, these two varieties differ with regard to the rate of canker development once infection is established, cankers in President extending more rapidly than those in Duarte. Periderm formation, moreover, starts earlier in Duarte than in President (tables 5 and 8). Hence the above generalization apparently holds true for all varieties tested.

The inference from this discussion is that periderm may possibly play a part in limiting canker activity. Earlier work (*16*) had suggested this possibility inasmuch as a concurrence between canker development and response of the host to presence of the disease was found to exist. When, for example, inoculations were made in early autumn at a time trees were capable of burying affected areas beneath new tissue, as described in the first part of this section, small lesions resulted. If, on the other hand, inoculations were made in late autumn, when the trees had lost the ability to react to the disease, larger cankers developed. As we have seen, the results of inoculation throughout the two years reported herein (figs. 1, 2) conform with those published earlier (*16*). In view of the coincidence between beginning of canker activity in autumn and loss by trees of their ability to produce periderm, records concerning stage of host development were kept each time the inoculations in figures 1 and 2 were made. In general these records show that larger cankers were secured from inoculations in autumn after the leaves had fallen than before leaf fall. In spring, maximum canker extension occurred sometime between the early blooming and first-leaf stages of the trees. The data concerning the first periderm formation in Duarte (table 6), the variety used in these inoculation tests, show that by early bloom stage this phenomenon was underway; that is, sometime before blossoms opened the trees regained their ability to produce periderm. The period between the time in autumn when the trees lost this ability and the time in spring when they regained it, corresponded roughly to the period when the disease may actively develop. Since data in figures 3 and 4 were interpreted to mean that some factor or factors tended to check canker extension even before the maximum springtime extension occurred, we may hypothetically assume that one factor, at least, was the regained ability of the host to produce periderm. Early in the spring, when the tree first begins to grow, the vigor of periderm formation is low; but it increases as spring advances and as the trees come into leaf. Such a conception answers the requirements of the evidence that influence of the factor towards limiting canker extension becomes more profound as spring advances. The as-

sumption that periderm may prevent extension of diseased areas presents nothing new. It has been investigated by Shaw (*11*) in the fire blight disease. This worker reports that the "cork layer" developing in advance of fire blight cankers appeared to prevent canker extension. When the layer was broken by mechanical means, cankers proceeded to extend even though they had previously ceased activity. Neither is the evidence presented herein, regarding activity of cankers during only certain parts of the year, unsupported by other workers. Wormald (*19–22*) could not obtain cankers of the cherry bacteriosis by inoculating in summer, but did obtain them when he inoculated at leaf fall (October or November). He further reports (*20*) that cankers stopped elongating in June.

If periderm causes waning canker activity in late spring and if, as shown earlier, phellogen activity depends upon growth of the tree as a whole, then cankers should cease activity earlier in those varieties which are first to begin growth in spring. Although no experimental evidence is at hand, the following observations are pertinent. Records taken in May, 1935 and 1937 (table 7), purported to show that cankers among trees of certain varieties differed with regard to the presence, absence, and relative number of dormant streaks. These streaks, it was found, were characterized by the fact that periderm had formed between the infected and the healthy tissues. More cankers in trees of early varieties possessed dormant streaks in considerable numbers than in trees of late varieties. In discussing the stages of canker activity (in the first section of this paper) the occurrence of dormant streaks was shown to indicate cessation of canker extension. Hence the data in table 7 may be regarded as evidence that by May a larger percentage of cankers were inactive in early than in late varieties.

To study more critically the connection between periderm formation and the waning of canker activity, numerous inoculations were made in May, 1937, into trees of Duarte plum, President plum, and Bing cherry, when the trees were in full leaf. The earlier experiments (figs. 3, 4) had indicated that cankers would develop more rapidly the first few days after inoculation than in subsequent days. To determine this point, 25 to 30 cankers were measured in each of the different species of trees 5, 8, 11, and 15 days after inoculation. At the same time pieces of bark at the apices of the cankers were taken for microscopic examination. To obtain a quantitative expression of periderm formation, freehand cross sections of the apices of 10 cankers were examined each time. The number of cells in the periderm, including the phellogen itself, were counted at several places around the invaded areas. This step was necessary because the layer did not develop uniformly on all faces of the canker: it frequently

appeared on the inner face (nearest the cambium) sooner than on the outer face (nearest the cortex). It also appeared earlier at the sides than at the apical margins, particularly if the diseased area at this point had extended inward to the cambial zone. Since, however, the information desired concerned the time when phellogen appeared at the advancing apical margins, this region only was considered in compiling the data.

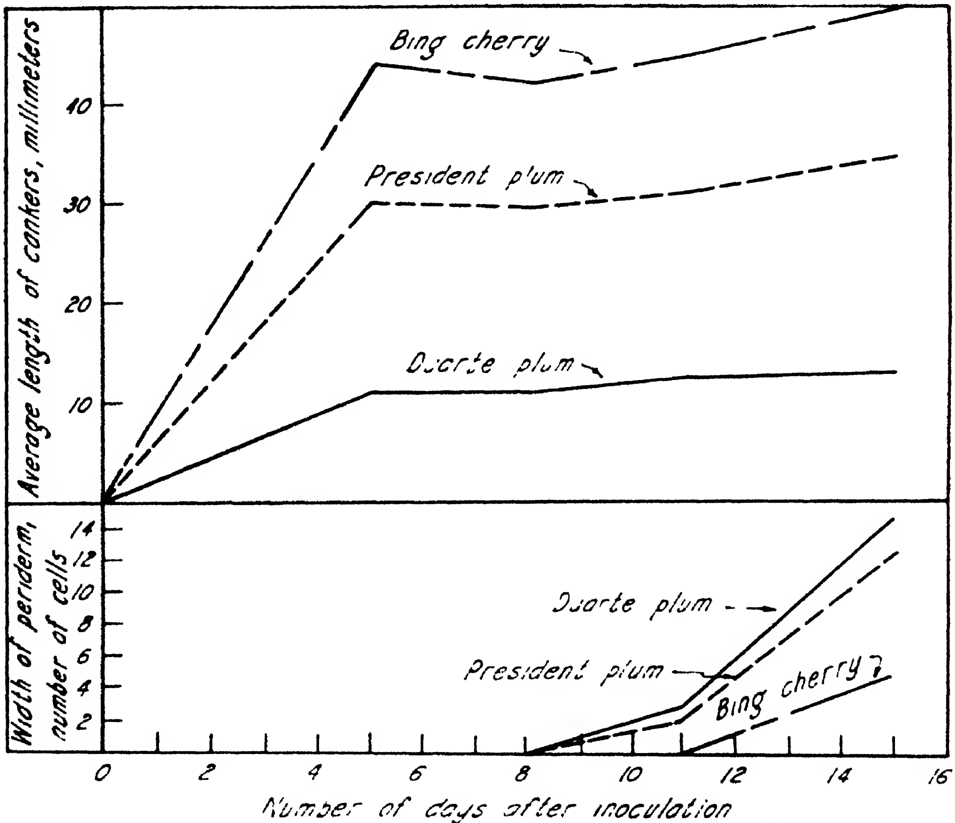

Fig. 9.—Comparison between canker enlargement and periderm formation in two varieties of plums and one variety of sweet cherry. Inoculations were made in May, 1937.

As with most former inoculations (fig. 6) the Duarte plum developed the smallest cankers, the President plum somewhat larger ones, and the Bing cherry the largest (fig. 9). Canker extension was very rapid in the three hosts during the first 5 days after inoculation, but very slow thence to the end of 15 days. These results accord well with those appearing in figures 3 and 4, wherein the rate of canker extension during the 10-day period after inoculation was more rapid than that for the second 10-day period.

Whereas rate of canker extension suddenly decreased in the present experiments 5 days after inoculation, periderm formation was not observed in the plums until 8 to 11 days after inoculation, nor in the cherry until 11 to 15 days after inoculation. True, the relative amount of periderm formed in trees of the three species was roughly proportional to the size of cankers produced. We should not, however, consider this as proof that formation of this layer prevented canker extension, since (fig. 6) cankers developing in Duarte plum and Bing cherry varied in the same relation when inoculations were made in January, at a time when we should not expect periderm formation. Before concluding, however, that no causal relation exists between periderm formation and cessation of canker extension, we should not overlook the possibility that some change in the tissue destined to become meristemmatic might precede appearance of the phellogen, and that such a change might check canker extension.

SUMMARY AND CONCLUSIONS

Criteria, involving visible changes in the characteristics of the canker margin, have been developed to determine rise and fall in canker activity. By these criteria activity was seen to begin in late autumn, continue throughout winter and spring, and halt in early summer.

Increases and decreases of the bacterial population in affected tissue accompanied rise and fall in canker activity.

Marginal characteristics of the cankers, though denoting to some extent cankers that will or will not constitute oversummering sources for the bacteria, are not entirely reliable, since other factors influence survival of the bacteria in diseased tissue.

By inoculating trees at intervals throughout most of two years, it was found that the period in the autumn when cankers could be obtained corresponded with that during which naturally occurring established cankers began activity. Likewise the period in spring when cankers could no longer be obtained corresponded to the period during which naturally occurring established cankers cease activity. Apparently these data verify earlier conclusions that the enlargement of the diseased areas is confined to certain seasons of the year. A similar seasonal activity is typical also of the pear canker caused by the same organism.

Experiments were performed to determine how various factors, including those of the external environment and those within the trees, affect the activity of cankers. Low temperatures during midwinter were seen to result in decreased canker extension, whereas the rise of temperature in spring was accompanied by increased extension. The failure

to obtain cankers by inoculating in early autumn and early summer was not, however, as far as the data warranted, attributable to adverse temperature. Especially, differences in rates of canker extension between two successive periods following inoculation could not be attributed to temperature differences. These data suggested that some factor began exerting an influence during the spring period when canker extension was at its maximum and that this influence became more pronounced as spring passed.

Moderately diseased trees growing on sandy loam soil were benefited by fertilization with ammonium sulfate. The benefit did not arise from increased resistance of trees to infection, nor apparently from increased resistance to extension of cankers through the tissues once infection was established, but seemingly from an enhanced ability of the tree to repair damages done by the disease.

Different soil-moisture conditions did not make trees growing in the field more or less favorable to progress of the disease. In tank experiments, however, where trees were located in soil lacking in available moisture—that is, soil in which the moisture had been reduced below the permanent wilting percentage—inoculations failed to induce cankers, whereas cankers were induced by inoculations into trees growing in soil with moisture above the permanent wilting percentage. The different results obtained in the two types of experiments are attributed to dissimilar conditions. That is, the orchard trees in the dry plots were probably never subjected to such extreme lack of available moisture as were trees in dry tanks, but may have been supplied with water by a few roots extending somewhat deeper than the 6-foot depth at which the samples were taken. The results can be interpreted as indicating that a lack of available soil moisture affected the disease adversely, whereas wide differences, short of an actual lack of available moisture, did not influence the disease. These studies by no means exhaust the possibilities of the problem as it relates to soil moisture.

Severity of the disease in a locality will be conditioned by the varieties grown. A few varieties are resistant, more are highly susceptible, but the greatest number are intermediate. Distinction is made between susceptibility to infection and susceptibility to inroads of the cankers once infection is established. One variety, the Duarte, classed as highly susceptible on the basis of tree mortality, proved consistently less favorable to progress of the cankers than another variety (President) which in two years suffered less from the disease. The former variety was highly susceptible to infection through buds, a situation apparently accounting for the high mortality of trees in certain years.

Internal reactions of the host to presence of diseased areas were considered from the standpoint of possible effect on progress of cankers. These reactions are induced by (1) the vascular cambium, or at least by cells in that region, and (2) by a cork cambium or phellogen. Major aspects of development of the phellogen and resultant periderm around the diseased areas at canker margins are described. Relation of growth activity of the tree to periderm formation is considered. This and the similar, though probably not identical, phenomenon of callusing of wounded surfaces were shown to depend upon growth activity of the tree. Such varieties of plums as Beauty, Kelsey, Santa Rosa, and Duarte, that began growth early were found to develop periderm around diseased areas and callus at surfaces of wounds earlier than such varieties as President, Grand Duke, and Tragedy, that began growth late.

According to certain observational data, cankers in early-blooming varieties of plums (Beauty and Santa Rosa) stop activity earlier in spring than cankers in late-blooming varieties (President, Grand Duke, and Tragedy). Experimental data indicate a certain relation between occurrence of periderm and cessation of canker extension, but fail to prove that the periderm actually prevented canker activity. This point is worthy of much additional work.

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SOME FACTORS AFFECTING THE SUSCEPTIBILITY OF PLANTS TO FIRE BLIGHT

H. EARL THOMAS AND P. A. ARK

SOME FACTORS AFFECTING THE SUSCEPTIBILITY OF PLANTS TO FIRE BLIGHT[1, 2]

H. EARL THOMAS[3] AND P. A. ARK[4]

STUDENTS OF FIRE BLIGHT (caused by *Bacillus amylovorus*) for a century or more (*4*)[5] have made observations which suggested that the environment of the inherently susceptible plant has a marked influence on the degree of susceptibility in any given situation. Certain elements of the environment have been studied exhaustively in more recent times, while others are still somewhat neglected.

In the work reported in this paper, consideration has been given to some of the factors which might be expected to affect the degree of susceptibility of a given plant, and more particularly those which might give some clue as to the nature of resistance to this disease. A few of the points dealt with have to do rather with escape than true resistance.

MORPHOLOGY OF THE PLANT IN RELATION TO INFECTION

Apart from wounds made by insects and other agencies, the entrance of the blight organism into the plant seems to be limited largely or entirely to natural openings, particularly in the nectaries and to a less extent in certain other flower parts (*31*), the stomata of the leaves (*14*, *30*), and probably also on the very young fruits (*26*) and shoots. It now seems probable (*8*, *17*, *36*) that penetration from the nectary into the receptacle, at least in the pear and apple, is through openings similar to or identical with the stomata.

There is a persistent impression, however, among pear growers and others, that infections may develop in the older bark of trunks, crotches, or large branches in the absence of any discernible shoot, spur, or wound. With this in mind, in three experiments, pear branches of varying ages up to about seven years were cut into convenient lengths and kept in a highly humid atmosphere until the whitish parenchyma tissues began to be exserted at various places on the surface. The blight organism was then applied to these tissues with a camel's-hair brush. No infection fol-

[1] Received for publication July 26, 1938.

[2] Assistance was received in parts of this work from L. Daniels, N. M. Heisinger, and B. Steele, students in the University and employees of the National Youth Administration, and from the federal Work Progress Administration.

[3] Associate Plant Pathologist in the Experiment Station.

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[5] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

lowed. Under these conditions, however, the killing produced by inoculation through needle punctures was slight (up to 2 mm from the point of inoculation in 10 days). Nevertheless, if the long experience of many workers in the orchard is included as evidence, it seems probable that the organism seldom if ever penetrates the bark directly in the absence of wounds.

Nixon (*29*) has pointed out that the path of migration of the bacteria within the plant usually lies in the outer cortical tissues which have large intercellular spaces, and some workers infer that the size of the spaces

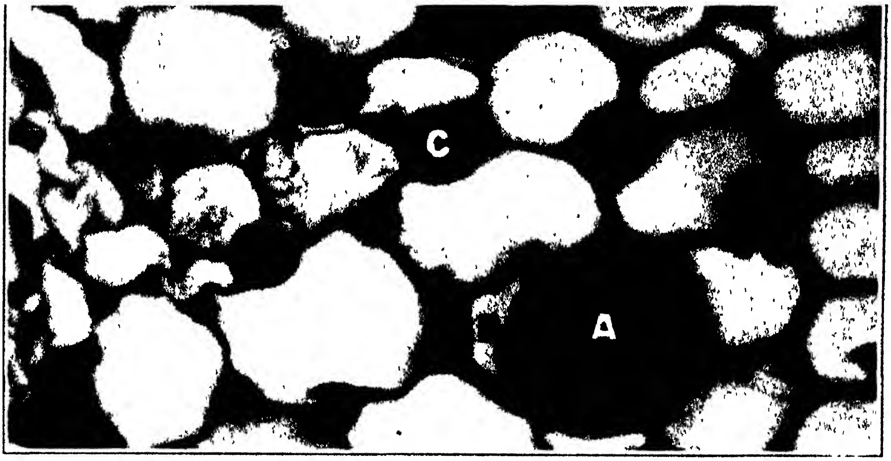

Fig. 1.—Parenchyma in midvein of leaf of *Pyracantha angustifolia* showing bacterial masses of various sizes in the intercellular spaces as at *A* and *C*. The cell walls are giving way, apparently under pressure from the masses, and at *B* a cell is almost entirely collapsed but apparently not yet invaded.

is a factor in determining the region to be invaded. The fact of selection of these tissues is unmistakable: in the older pear branches, the organism may advance for at least 12 inches and cease activity without killing the inner bark. To study this point further, transverse sections were cut from stems of the susceptible *Cotoneaster pannosa* about ½ inch from the growing tip and again about 2 inches from the tip. The latter would represent distinctly less-susceptible tissues. However, the intercellular spaces here were much larger than in the younger tissues. Sections were also prepared from comparable younger tissues (½ inch from the tip) of the virtually immune *C. Franchetii*. In these, 20 of the larger spaces in the parenchyma chosen more or less at random (by an assistant) averaged 35 by 82 microns, whereas 20 corresponding spaces in the younger tissues of *C. pannosa* averaged 27 by 53 microns. While it seems to be true in general that the larger spaces are in the tissues traversed by the migrat-

ing masses of bacteria (zoogleae) (*29*), the facts just given indicate that the size of intercellular spaces is not a primary controlling factor in the movement of the organism in the plant. This view is further supported by a study in sections of tissues recently invaded by the organism (fig. 1). These show, as Bachmann (*3*) has pointed out, that the spaces are readily enlarged in response to the pressure or some other influence of the zoogleae. Thus the bacteria may come to occupy almost the entire space formerly occupied by a cell without having invaded the cell. Furthermore, in cross sections of invaded stems the spaces actually occupied by the bacterial masses are often much smaller than others nearby which are not occupied (*29*).

The interesting alignment of the bacterial cells parallel with the line of advance reported by Nixon (*29*) and Haber (*12*) has been observed in our sections, but only in intercellular spaces which are relatively long and narrow. Probably this alignment is an instance of the general physical phenomenon of orientation due to laminar flow observable when rod-shaped particles suspended in a fluid are passed through a tube of small diameter (*10*). This point is of interest as an additional indication that the organism is nonmotile in this phase (*29*) and merely carried by the moving matrix.

HUMIDITY IN RELATION TO INFECTION

Although earlier work (*7*) indicated that the optimum atmospheric humidity for infection was about 80 per cent, the more recent and exhaustive work of Shaw (*38*) has shown beyond reasonable doubt that an atmosphere near saturation is most favorable. This fact is perhaps responsible in some measure for the seemingly excessive emphasis which has been placed upon water as an agent of dissemination (*26*).

The results with the different methods of inoculation are no doubt related to humidity or desiccation (see also section on age of wounds, p. 310). On several occasions, apple and pear trees in both New York and California were inoculated in wounds of various kinds. These experiments may be sufficiently represented by one made on Bartlett pear near Walnut Grove, California, on May 21, 1930. The weather during this period was relatively dry. Twenty-five current-season shoots and 25 branches two to three years old were cut off with pruning shears, each cut being preceded by a cut through an active canker. In a third lot, 25 wounds were made by cutting off the outer bark with a sharp knife; the exposed surfaces were then smeared with a water suspension of the bacterial exudate. Finally, 25 ragged wounds were made by gouging into the bark with the point of a knife and inoculated as in the preceding

lot. In these four lots, respectively 2, 2, 0, and 1, infections resulted. Of 10 shoots inoculated near the tips at the same time by puncturing with a needle dipped in the bacterial suspension, 9 became blighted.

Studies of nectars (*6*) and of cultures containing varying concentrations of the sugars found in nectars (*41*) have shown that the concentration of sugars in the nectar is inversely related to atmospheric humidity and very probably limiting to the penetration of the blight organism into the plant at low atmospheric humidities.

Hildebrand has recently concluded (*15*) that Thomas and Ark (*41*) "probably have exaggerated the importance of nectar dilution by rain in bringing about blossom infection, because other openings than those found in the nectary, and particularly the stigmas, were left out of consideration by them as infection courts," and that "only in the pear, which has an open receptacle, would the desiccating influence of the atmosphere be strongly inhibitive." Several pertinent facts seem to have been overlooked in arriving at these conclusions. First, the fluctuations in atmospheric humidity rather than direct dilution of the nectar by rain is the factor believed to influence greatly the inception of infection; second, the concentration of the nectar in the plants with sheltered nectaries has been shown to be readily altered by changes in atmospheric humidity, although somewhat less rapidly than is the case with plants having more exposed nectaries (*5*)[6]; and third, Pierstorff (*31*), working in New York in association with one of the present writers, studied in some detail the infection of stigmas and other flower parts of pear and apple blossoms as early as 1925 and 1926 and reached the conclusion, probably still valid with respect to petals and stigmas, that "under field conditions the number of infections resulting from the entrance of bacteria through these infection courts is small."

After penetration has taken place, the concentration of the sap as well as humidity per se (*38*) may possibly be an important controlling factor, although since these are no doubt to a considerable degree in inverse relation, the complete separation of these factors does not seem possible at present. The osmotic pressure of sap of apple leaves has been shown (*21*) to vary from between 7 and 9 atmospheres at a time of heavy rainfall and low temperature to between 20 and 23 in relatively dry periods. A similar change in Bartlett pear leaves at Berkeley from 11.7 atmospheres on April 2 to 24.2 on August 4 has been noted by J. P. Bennett (unpublished). Much wider fluctuations in osmotic values have been

[6] In one of our own tests, apple blossoms on branches kept overnight in an atmosphere near saturation yielded nectar with a sugar content of 6 to 7 per cent, while blossoms similarly treated except exposed to the laboratory air contained 35 to 44 per cent sugar.

reported for the desert plant *Larrea tridentata* (*25*), only part of which are apparently referable to changes in moisture supply. Apparently these agencies are less sharply limiting after infections are well established, as evidenced among other things by the "water-soaked" condition of the tissues at the margins of active cankers and the plasmolysis of cells (*3*) in early stages of infection. The production of a toxin capable of wilting susceptible shoots has been demonstrated (*31*) for the blight organism. Interference with the water retention in plant cells by materials toxic to the tissues seems to be a rather general phenomenon (*18*).

The relation of soil water to blight infection has not been extensively studied. In experiments in the greenhouse with potted plants (*42*), maintaining the water content of the soil near the wilting point caused a striking reduction in the extent of infections. Apple and pear trees grown in soil with controlled water content at 40 to 50 per cent of capacity and apparently never allowed to reach the wilting point have been shown (*37, 38*) to possess greater resistance than trees grown at 80 to 90 per cent. In a field experiment at San Jose, several hundred three-year-old seedling pear trees were inoculated in the trunks 4 to 6 inches below the ground line. The soil was replaced and part of these were irrigated within the next 24 hours so that the soil around the points of inoculation was wet. This was done in the middle of the dry season, July 16, and the number infected was low in both lots, 8 of 100 in the irrigated rows and 6 of 50 in the rows not irrigated at that time.

In small-scale tests, the application of slightly increased water pressure to the roots of *Cotoneaster Franchetii* and to the stems of *Pyracantha angustifolia* produced no perceptible change in the resistance of the former or the degree of susceptibility of the latter, which blighted to 2.3 inches from the tips in 4 treated plants and 2.4 inches in 4 control plants. The pressure on the latter species was obtained by a column of water 8 feet above soil level and on the former by a somewhat shorter column. Two large potted plants of *P. angustifolia* were subjected to controlled water pressure of 20 and 28 pounds beginning at the time of inoculation and continuing for 4 and 5 days respectively, without perceptible increase in susceptibility. A plant of *Cotoneaster glaucophylla* similarly exposed to a pressure of 20 pounds remained immune. There was no visible "water-soaking" in any of these treated plants. Considerably higher water pressures have been shown (*20*) to increase enormously the susceptibility of tobacco and other plants to bacterial pathogenes. In view of the relatively high internal pressures which plants seem capable of developing (*44*), perhaps the addition of a few pounds should not be expected to produce any marked change.

RELATION OF TEMPERATURE TO INFECTION

Some indirect effects of temperature on fire blight have already been pointed out (*42*). Evidence from many sources indicates that blight infection may be active over a wide range of temperatures but is favored by relatively high temperatures. This is well illustrated by the destructiveness of the disease in the more humid areas of the southern United States. On the basis of controlled experiments, the optimum temperature has been placed between 70° and 80° F (*7*). The optimum temperature for the bacteria in culture seems to be about 83° F (*2*). There is probably no upper limit of temperature under natural conditions. The bacteria in blighting shoots $\frac{1}{16}$ to $\frac{1}{4}$ inch in diameter are able to withstand external air temperatures of 4 hours at 118° F, or 30 minutes at 140° F (*42*). On the other hand, low temperatures are known to retard or arrest the development of infections. In areas with mild winters, direct evidence can be seen of temperature as a limiting factor in the enlargement of cankers. Those cankers which have entirely girdled the branches are often found to advance several inches more during the winter months on the side exposed to the sun than on the opposite side. A relation of low temperatures to initiation of infection is also indicated by results of an experiment in which alternate rows of seedling pear trees, $\frac{1}{2}$ to $1\frac{1}{4}$ inches in trunk diameter, were inoculated on the north and south sides of the trunks at San Jose, February 19, 1936. On April 7, 15 per cent of 73 trees inoculated on the north side and 28.5 per cent of 56 trees inoculated on the south side were infected.

RELATION OF LIGHT TO INFECTION

The effect of heavy shading or complete darkness on infection has been briefly reported (*42*) and, since it was slight, will not be treated in detail. The etiolated shoot tips of shaded plants blighted somewhat more rapidly, but the stems developed on these plants before the experiment began were less affected. Thus, in one experiment with pear seedlings, the organism penetrated to or into the preceding growth cycle in 12 of 20 shaded plants and 17 of 20 controls in 11 days.

NUTRITION AND GROWTH STAGE IN RELATION TO INFECTION

The close relation between susceptibility and the vigor of the tree has long been a matter of common knowledge. Even though several workers (*7, 16, 28, 37, 39*) in more recent time have presented experimental proof, this factor is not always given adequate consideration.

The relation of nitrogen supply to infection is readily seen in tests with root-bound plants of *Pyracantha angustifolia.* In one of several such experiments, 20 plants in 6-inch pots of poor soil were selected in pairs, and one of each pair received ¼ gram of calcium nitrate. All were inoculated at the tip 5 days later, and the length of the blighted part measured 13 days after inoculation. The average for those with added nitrogen was 5.9 inches (5.0 to 7.2) and for the untreated 2.2 inches (1.5 to 3.2). In this experiment, all the plants became infected; but in others, the number of infections, as well as the rate of extension, was increased by addition of nitrogen. Thus plants of the same species were given calcium nitrate in 3 of 6 flats, each treated flat receiving 2 applications of 5 grams with an interval of 5 weeks between. All were inoculated 5 weeks after the last applications. Eleven days later, 62.5 per cent of 176 plants with added nitrogen were blighted, as compared with 40.0 per cent of 190 plants in the untreated flats.

That the young, actively growing parts of a plant are in general much more susceptible than older parts even only a few inches distant, is so well known as to hardly require mention (*42*). A few exceptions, however, seem to have been noted (*34*). Observations in orchards and on several thousand seedling trees inoculated in the trunks at different times indicate that the bark of the trunks and main branches of pear trees is more susceptible at the end of the growing season than in midseason or earlier; but this may be due to the higher relative humidities usually prevalent in autumn.

As distinct from the nutrition of the suscept may be mentioned that of the pathogene in artificial cultures, which has recently been shown (*2*) to increase or decrease the virulence of the organism according, among other things, to the concentration of sugars in the medium.

It has also been suggested that arbutin (*31*) and asparagin (*2*), which are utilized by the organism in cultures and believed to be present in the actively growing parts of the pear or apple (*24, 28, 43*), may bear some relation to susceptibility. With arbutin in mind, inoculations were made (at Ithaca, New York) on shoots, fruits, and leaves of the following, at least some of which are considered to be rich in arbutin: *Arctostaphylos Uva-ursi, Epigaea repens, Gaultheria procumbens, Vaccinium vacillans.* No infection followed.

In the case of asparagin, Ark (*2*) has already reported that additions of this compound to the inoculum or injection into the plant seemed to accelerate infection in comparatively resistant tissues. More recently, inoculations have been made on 12 species of rosaceous plants with and without asparagin added to the inoculum (a water suspension or, in one

case, beef-peptone broth). There was a small but consistent increase in the extent of disease in plants which are susceptible under ordinary conditions. For example, 20 paired shoots of *Pyracantha angustifolia* were inoculated, half of them with asparagin added to the inoculum. The latter averaged 2.22 inches in length of blighted shoot after 14 days while the controls averaged 1.65 inches. The presence of asparagin did not induce susceptibility in plants which are ordinarily immune or nearly so, such as *Cotoneaster Franchetii, C. glaucophylla, C. Harroviana, C. lactea,* and *Rosa Soulieana.*

WOUNDS AND WOUND REACTIONS IN RELATION TO INFECTION

Wounds are of interest, not only as portals of entry of the organism into the plant, but for the further reason that the margins of infected areas seem to be in certain ways comparable to those of wounds caused by other agencies. Moreover, wounds which interfere with the movement of nutrients may alter the susceptibility of adjacent tissues.

Girdling.—In mid-June 1930, 184 Yellow Newtown apple trees were girdled on the upper trunks by the Department of Pomology of Cornell University at Ithaca, New York. This was done by cutting through the bark to the wood without removing any of the bark. Of these trees, 64 developed blight starting at the ring; 81 per cent of the cankers extended farther above than below the ring, and 65 per cent were limited to the bark above the ring. Only 7 per cent of the cankers were entirely below the ring. A similar observation from the same orchard has recently been reported (*16*).

In the case just cited, inoculation may have been effected at the time of girdling or, as seemed more likely, within the next few days (by insects). An experiment was therefore devised to test the effect of girdling when the inoculum was applied at the time of wounding. For this purpose, several score of seedling pear trees were partially girdled at San Jose in June, 1935, by cutting through the bark around one-fifth to one-third of the circumference of the trunk. A roll of cheesecloth was clamped to the back and sides of the knife used in making the cuts and this was kept moist with the inoculum. Thirty-seven infections were found 6 weeks later; 73 per cent of these extended farther above than below the ring while 24 per cent were larger below the ring. The average length of the cankers was 3.27 inches above the ring and 1.75 inches below.

Several experiments in some respects related to the girdling of stems were made on potted plants of *Pyracantha* and *Cotoneaster* in the greenhouse. In these, 2 to 4 inches below the points of inoculation (tips of

shoots), wounds were made by puncturing the stem once or twice with a fine needle, by constriction with rubber bands, or by abrading the surface with powdered carborundum. It was assumed that these treatments would not greatly interfere with translocation but might cause the neighboring tissues to develop some substance unfavorable to the passage of the blight organism. The effects of such wounds were slight. The length of blighted part was somewhat greater on wounded shoots than on comparable control shoots in three of four experiments but not significantly so in any one of them. In a total of 72 wounded shoots of which 64 became infected in seven tests, the infection stopped at the points of wounding in 13 but advanced beyond these points in 29 others.

TABLE 1
RELATION OF DEFOLIATION TO INFECTION IN SHOOTS OF PYRACANTHA ANGUSTIFOLIA

Experiment	Interval from defoliation to inoculation	Treatment	Number of plants	Average length of blighted part
	days			*inches*
1	7	Defoliated	8	5 09
		Controls	8	8 56
2	6	Defoliated	6	0 45
		Controls	6	3 95
3	3	Defoliated	10	2 30
		Controls	10	3 75

Removal of Growing Points.—To determine whether the growing tips of shoots have any important influence on infection, a single experiment was made in which 10 plants of *Pyracantha angustifolia* were inoculated about 1¼ inches below the tips, and the tips of 5 were cut off at the same time. All became infected, and after 10 days the average length of blighted shoot was 3.2 for the treated and 3.4 inches for the control plants.

Defoliation.—In blight-control work the infections which are overlooked, especially near the distal ends of heavily pruned branches, often seem to be retarded for some time afterward. The case (*1*) may be cited here of an orchard in Lake County in which the trees were severely affected by blight in the spring of 1934. A number of these trees were almost completely defoliated in April, 1934, by heading back many of the main branches and cutting off most of the spurs and small branches. Several competent observers agreed that the development of infections on these trees was stopped distinctly earlier than on similarly infected trees in the same orchard on which the cutting was much less drastic.

These and other observations prompted several experiments (in the greenhouse) designed to test more specifically the relation of defoliation to infection. In the first of those represented in table 1, all of the leaves were removed from the treated plants. This resulted in so obvious a decline in the plants that in the two later experiments the leaves were removed from the upper half of the stem only. All the plants became infected except 3 defoliated in experiment 2. The plants of this test were root-bound and in rather low vigor, which no doubt accounts in some measure for the greater difference between treated and control plants in this experiment.

TABLE 2

RELATION OF AGE OF WOUNDS AND HUMIDITY* TO INFECTION IN SHOOTS OF PYRACANTHA ANGUSTIFOLIA

Experiment	Age of wounds	Number of shoots	Number of infections
	hours		
1	27	5	0
	0	5	5
2	6	15	3
	0	15	13
3	6	15	8
	0	15	10
4	6	15	8
	0	15	8

* See text, below.

Wounds of Different Ages as Infection Courts.—Previous work (*7*, *42*) has shown that wounds in leaves and stems of the apple and the roots of pear trees become closed to invasion by the blight organism within 72 hours. Some additional experiments with shoots of *Pyracantha angustifolia* are summarized in table 2. The shoots were wounded by single needle punctures near the tips, and the bacteria were applied to the wounds in water suspension with a camel's-hair brush immediately after the last wounds were made in each experiment. The plants of the first and second experiments were kept in the greenhouse throughout; those of the third were kept in a moist chamber for 4 days beginning just after inoculation, so that the first set of wounds were exposed to dry air for 6 hours before inoculation; and those of the fourth for 5 days beginning when the first plants of this experiment were wounded. The results indicate that wounds became unfavorable to infection more quickly than has been supposed and that humidity is important but perhaps not the only cause of the differences shown in the table.

Direct tests were made on sections of bark adjacent to wounds of different ages for the presence of gums, pentosans, suberin, and other substances, chiefly by the methods outlined by Rawlins (*33*). The reagents used most extensively were orcinol, phloroglucinol, and Sudan IV. The wounds were made by cutting longitudinal slits in the bark of growing

Fig. 2.—The formation of a cicatrice in blighted pear bark beyond the margin of the dead bark.

shoots. At least 16 species of plants in the genera *Cotoneaster, Eriobotrya, Photinia, Pyracantha,* and *Pyrus* were used. These provided a range from highly susceptible to completely immune individuals.

There was no evidence of a relation between susceptibility and any of the staining reactions which were tested. For example, the apple (*Pyrus malus*) and pear (*Pyrus Kawakamii*), which are susceptible, gave a suberin reaction on the third day from the time of wounding and so did *Cotoneaster Franchetii* and *C. Harroviana* which are virtually immune. The susceptible loquat (*Eriobotrya japonica*) gave no positive reaction within 5 days with any of the three reagents mentioned above.

Occasionally among seedlings of the Surprise pear there appear individuals with thick, corky bark on parts only two or three years old. One such tree was propagated and later inoculated in the greenhouse. This did not seem to be more resistant than the average pear seedling.

That some factor other than the suberin which eventually appears in the cicatrice, probably operates to arrest the advance of the infection, is suggested by the fact that the cicatrice seems to form after the advance has stopped, at least temporarily, and frequently at some distance beyond the apparent margin of the infection (fig. 2). Shaw (*37*) has shown, however, that the cicatrice does oppose a barrier to the renewal of advance from an arrested infection.

POSSIBLE INHIBITORY SUBSTANCES IN THE PLANT

Mature fruits of pear and apple are much more resistant to blight infection than young fruits of the same variety. Elmer (*9*) has shown that ripe apple fruits in a closed container give off a volatile substance capable of inhibiting the growth of potato shoots. With these facts in mind, four tests were made with ripe apple and one with pear fruit in which slices of the fruit were placed inside the covers of inverted petri-dish cultures of the blight organism. These were incubated at 25° and 28° C. The organism grew vigorously in all such cultures and apparently fully as well as in control cultures.

A partial parallel between susceptibility and the anthocyanin pigment in the bark of pear seedlings has been reported (*40*). Further tests were made bearing on this problem with the lots of hybrid *Pyracantha* seedlings treated in the succeeding section of this paper. In two of these lots, 20, and in the third, 25, seedlings were selected showing the most pronounced red pigment which could be found, and a like number in each lot showing the least red pigment. The numbers of infections in the first lot were 6 red and 11 green, but the corresponding figures for the other two lots were 14 red to 12 green and 14 red to 8 green. There was no marked difference in average extent of the infections between the two groups in any of these tests. The last two tests were made in a heated greenhouse near midwinter. The red-stemmed plants were not strongly pigmented under these conditions and seemed to be somewhat more vigorous than the green plants.

In a further test with pear, shoots from the stocks of older orchard trees were selected in contrasting groups according to the amount of red pigment in the bark at the time of collection. These were bench-grafted on seedling roots, grown in the field for two seasons, and inoculated at the tips of shoots in the spring of the third season. The contrast in color

did not persist under these conditions and the 39 surviving plants selected as "red" blighted quite as readily (69 per cent) as the corresponding 35 "green" plants (60 per cent) blighted. Probably the red pigment is related to blight resistance only in so far as it is associated with unfavorable growth conditions of the plants or in some other indirect way.

The toxicity of phenolic substances to bacteria in general is well known, and several of them have been tested against the fire-blight organism. The following are toxic to the organism in the concentrations indicated: hexylresorcinol $1:10^3$, hydroquinone $1:10^5$, pyrocathechin $1:10^3$, quinine sulfate $1:6 \times 10^3$, thymol $1:10^3$, cadmium salicylate $1:10^5$, strontium salicylate $1:10^6$. Hydroquinone is known in rosaceous plants (*43*), as in many others, but the relative amounts in different species do not seem to have been studied in detail.

Various materials of some interest in this connection have been used from time to time, either as drenches on blight cankers in pear orchards or as sprays on inoculated seedling trees. Lithium, methyl, and sodium salicylates when applied to the surfaces of active cankers in different solvents, consisting principally of alcohol and glycerine, had a definitely depressing effect on the progress of infection during periods of high temperatures. No such effect was clearly apparent in similar but small-scale tests with hydroquinone, pyrocatechin, and tannic acid, although there was some suggestion of it with the first of these.

Solutions in water of sodium salicylate (0.2 and 1.0 per cent), hydroquinone (1.0 per cent with 4.6 per cent glycerine), and glycerine (5 per cent) were each sprayed on several hundred seedling pear trees 4 days after these had been inoculated. Twenty days after inoculation, the average length of the blighted part in inches for 100 trees for each treatment in 6 consecutive rows was: control, 6.3; sodium salicylate 1 per cent, 5.3; sodium salicylate 0.2 per cent, 5.4; hydroquinone plus glycerine, 4.4; glycerine, 5.8; control, 6.8.

The question is sometimes raised as to whether a coating of bordeaux mixture or other spray material has any effect on the resistance of the sprayed plant. In two experiments of 12 and 24 paired plants of *Pyracantha angustifolia*, half were sprayed with bordeaux mixture 1–1–50 in the greenhouse and all were inoculated at the tips when new growth had appeared above the sprayed parts (about 3 weeks after spraying). In one of these experiments the plants were cut back and inoculated a second time 9 weeks after spraying. In all three inoculations the blight was slightly greater on unsprayed plants but not significantly so.

A similar test was made later on 46 paired plants of *Cotoneaster pannosa*. Again the blight was somewhat more active in unsprayed plants,

but the difference was even less than in the preceding tests, the average length of blighted part being 2.44 inches for sprayed plants and 2.63 for the controls.

Probably, with trees in the orchard, the difference in susceptibility due to spraying is slight if measurable at all.

INHERENT RESISTANCE

As far as is known, all the plants outside the rose family and many within it possess either a natural immunity to or a capacity to escape fire-blight infection. Even though all the genera in the subfamily Pomoideae (*42*) and some genera in all the other subfamilies contain susceptible plants, there are species or individuals in most of the genera, including *Pyrus*, which are highly resistant or immune. Resistance in the pear species and varieties has been of interest to many workers and has been studied extensively by Reimer (*34*).

Since many resistant root or intermediate stocks are now in use in pear orchards, the question sometimes arises as to whether these affect the susceptibility of the top varieties grown on them. The extensive planting of the highly resistant Old Home variety as an intermediate stock, usually on susceptible seedling roots, has provided abundant material for observation on this point. There is no evidence, however, of any change in the susceptibility of the top variety or of the roots, even, in the latter case, when the Old Home top is allowed to grow. Likewise when *Cotoneaster Franchetii* top (nearly immune) was grown on seedling pear stem and root in pots, the inherent susceptibility of the pear appeared to be unchanged up to more than one year from the time of grafting.

Seedlings of *Pyrus communis* vary widely in susceptibility to blight as in many other characters. This is illustrated by an experiment in which 500 seedlings from an Old Home × Farmingdale cross were planted at San Jose beside a like number of seedlings from trees of Winter Nelis and Beurre Hardy which had been exposed to Bartlett pollen. The varieties Old Home and Farmingdale are both highly resistant (*34*) although generally considered to be entirely of *P. communis* origin. Winter Nelis is among the more resistant of the commonly cultivated varieties, whereas Beurre Hardy is distinctly more susceptible, at least as to the extent of infections once established. The above seedlings were inoculated at the tips in April of the same year in which they were planted, with the results, as recorded 2 weeks later, shown in table 3. Although a few of the Old Home × Farmingdale seedlings were later killed entirely by blight, the difference in resistance between this lot and the other two

appeared to be greater after subsequent inoculations than is indicated by the table.

Something over 10,000 seedlings of Beurre Hardy and about 35,000 seedlings of Winter Nelis similar to those mentioned above have been grown for six years at San Jose with several inoculations each season. More than 5,000 of these have survived. It is not possible to state what part of this survival is due to inherent resistance and what part to crowding, dry weather, and other factors unfavorable to infection. However, the fact that a considerable number of the most vigorous trees in the planting have failed to develop appreciable if any infection suggests

TABLE 3

SUSCEPTIBILITY OF PEAR SEEDLINGS OF DIFFERENT PARENTAGE

Source of seed	Proportion infected		Length of blighted part	
	Number trees examined	Per cent blighted	Number examined	Average length
				inches
Old Home × Farmingdale	490	16.5	100	1.79
Winter Nelis	481	46.9	200	2.32
Beurre Hardy	500	45.6	200	2.71

that some of them are possessed of a high degree of inherent resistance. Reimer (*35*) has reported the occurrence of two highly resistant individuals out of 235 and 50 out of 704 in two lots of *Pyrus communis* seedlings.

The wide variability in any group of seedlings of pear or apple renders this material highly unsatisfactory for observations on the relation of inheritance to blight resistance. For this reason, tests were made with the progeny of a cross between *Pyracantha angustifolia* and *P. Gibbsii* var. *yunnanensis*. The former is highly susceptible to blight and remarkably uniform from seed. The latter is relatively resistant to blight, and while the seedlings vary appreciably, only an occasional plant is sharply different from its mates. The four plants which survived in the first generation from this cross were virtually identical in appearance and very similar to the seed parent *P. angustifolia*, in this respect resembling the recently reported behavior of pear hybrids (*19*). In resistance these were also very similar to each other but very near the pollen parent—directly opposed to the condition reported for pear (*19*).

Lots of seedlings were grown in pots to a suitable size for inoculation from three of the F_1 individuals[7]. These lots are designated as hybrids

[7] Pollination of these was not controlled. It was assumed on the basis of behavior of the parents that cross pollination was not likely to occur.

1, 2, and 3. At the time of inoculation, each lot was divided into four groups A, B, C, and D, including on one extreme (group A) the plants most resembling *Pyracantha angustifolia* in leaf characters and on the other (group D) those most like the other parent, with two intermediate groups between. Parallel with the inoculation of hybrid 2, inoculations were also made on shoots of the parent species (table 4). The results are

TABLE 4

INFECTION OF F_2 SEEDLINGS OF A CROSS BETWEEN PYRACANTHA ANGUSTIFOLIA AND P. GIBBSII YUNNANENSIS GROUPED IN RELATION TO RESEMBLANCE TO THE PARENTS

Plant	Group	Number of plants	Number blighted	Length of blighted part
				inches
Hybrid 1	A	20	7	2.1
	B	20	6	2 5
	C	20	12	1 6
	D	20	10	2 4
Hybrid 2	A	25	9	1 3
	B	25	7	0 8
	C	25	13	1 9
	D	25	11	1 8
Hybrid 3	A	20	14	1.6
	B	20	11	1 8
	C	20	12	1 9
	D	20	14	1 7
Pyracantha angustifolia	....	25	25	3 7
P. Gibbsii var. yunnanensis		25*	0	

* Twenty-five shoots on 7 large potted plants. In all others, only one inoculation per plant was made

fairly representative for *P. angustifolia* but are rather extreme for *P. Gibbsii* var. *yunnanensis,* which not infrequently blights for an inch or so at the tips of growing shoots after inoculation. As may be seen in the table, there is actually more blight in the C and D groups than in groups A and B. This seemed to be due to a somewhat lower state of vigor in the latter under the conditions of the tests; probably the differences are not otherwise significant. The results thus far suggest that resistance in this cross is at least partially dominant and, as in the pear (*40*), that resistance is not associated with any of the readily observable morphological characters.

DISCUSSION

In the present state of our knowledge of disease resistance in plants, any discussion of this subject must come to rest on incomplete foundations. Nevertheless, since resistance is at the core of many problems in plant pathology, it may be worth while to consider those facts, even though fragmentary, that seem to bear some definite relation to this phenomenon. There is available, at any rate, sufficient evidence to indicate that resistance in plants is not due to a common cause but may be due, even in a single disease, to several distinct causes (*11*).

There is some evidence to support the concept that resistance to the initiation of fire-blight infection through wounds is due to factors different in some way from those which terminate the course of infection. For example, in certain of the experiments reported in this paper, the proportion of plants infected may be distinctly different in two groups, while the average extent of the infections is essentially the same. This view is further supported by the apparent capacity of the blight organism, once established in the plant, to alter markedly the physiological processes of the invaded tissues, evidenced among other ways by the early plasmolysis of cells (*3*) and by the "water-soaking" of the bark, presumably due to the release of water into the intercellular spaces.

That there is some relation between susceptibility and the nitrogen content of the tissues is indicated by the relatively greater susceptibility and apparently greater nitrogen content in young, actively growing parts than in older parts of the same plant, in etiolated shoots than in shoots exposed to light (*23*), in plants with ample water supply than in plants with low water (*22*), in the tissues immediately above the point of ringing than in those below the ring (*13*), and in plants with added nitrogen than in plants without such additions.

If nitrogen per se is a determining factor, it would seem to be in the form of some particular compound or group of compounds rather than as total nitrogen. In spring when growth is active and susceptibility high, the amino nitrogen in pear bark is relatively high as compared with the basic forms, whereas during the dormant season, the reverse is true (*27*). Furthermore, defoliation, which is followed by an increase in percentage of total nitrogen in apple shoots (*13*, *32*), causes a reduction in susceptibility.

The vigorous growth of the pathogene in many and varied nonliving media, as well as in more than 125 species of rosaceous plants, suggests, however, that resistance is not due primarily to lack of nutrients in the plant but rather to the presence therein of some substance or substances

in quality or concentration deterrent to the pathogene. In additional support of this view may be cited the failure of nutrients added with the inoculum to increase infection to any considerable degree in susceptible plants or to break down the high resistance or immunity in other closely related plants. The fact, noted by many workers, that the organism dies out rather quickly in blighted bark may also be considered as evidence bearing on this point.

Specific evidence has been presented (*37*) indicating that an arrested infection in the apple is usually held in check (prevented from revival) thereafter by the formation of a cicatrice at or near the margin of the infection. Such barriers are, however, relatively slow in formation and seem to appear only after the advance has been retarded or stopped by other means. The failure in this work to find evidence of suberin in wounded tissues until long after these have become closed to invasion by the blight organism suggests also that substances other than suberin are involved in preventing entrance through wounds. These, however, may be precursors of suberin or related compounds.

El-Sawy has shown[8] that wounding causes increases in reducing substances of up to more than 50 per cent near the wounds in pear bark, and it is perhaps possible that these reach sufficient concentration to inhibit the organism under certain conditions. But quantitative determinations of these substances in the tissues immediately adjacent to wounds do not seem to have been made.

SUMMARY

Observations on the histology of the shoots of resistant and susceptible plants in general support those of earlier workers indicating that the size of intercellular spaces is a minor factor in determining the course of infection.

The tissues known to have a high nitrogen content are in general more susceptible than the nearest comparable tissues of lower nitrogen content.

The evidence suggests that the concentration of solutes in the nectar and perhaps also in the plant sap as affected by atmospheric humidity is of importance in the penetration of the organism into the plant and in the subsequent development of infection.

On the trunks of girdled pear and apple trees, the bark immediately above the point of girdling is more susceptible than that immediately below.

[8] El-Sawy, Abbas H. Some effects of wounding on respiration and food reserves in the pear tree (*Pyrus communis*). Typewritten thesis, University of California.

Slight wounds 2 to 4 inches below the points of inoculation of susceptible shoots did not cause any marked change in the development of infection.

Tests for gums and suberins failed to detect these substances at the margins of wounds until after the period during which the wounds would be susceptible to invasion.

Etiolation has a relatively slight influence on infection, while defoliation definitely reduced susceptibility.

In the progeny of a hybrid of *Pyracantha angustifolia* and *P. Gibbsii* var. *yunnanensis,* resistance seemed to be at least partially dominant. In the F_2 generation there was no observed relation between susceptibility and resemblance to the parents.

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THE USE OF CHEMICAL DATA IN THE PROGNOSIS OF PHOSPHATE DEFICIENCY IN SOILS[1, 2]

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LACK OF CORRELATION BETWEEN PLANT GROWTH AND ACID-EXTRACTABLE PHOSPHATE

SOIL CHEMISTS have long recognized that there is a certain rationale in extracting soils with acids to determine the capacity of soil particles for releasing ions to the plant. Unfortunately, attempts to correlate the actual amounts of given ions released by acids in the laboratory with the amounts of such ions absorbed by plants have not been highly successful.

Two general types of explanation might account for the discrepancy. The first of these is that, although the release of H ion to the soil by plants and microörganisms is always quantitatively important, the plant can, by absorbing from the liquid phase, shift the equilibrium between the liquid and solid phase without a material change in the H ion concentration of the system. Ions thus released independently of evolution of acid by the plant become a part of the soil solution and can be absorbed by the plant. Moreover, H. Jenny[5] has shown that the merging of the swarm of ions in the electrical double layer of the soil particle with a similar system at the root-hair surface, is sufficient to cause the removal of an exchangeable cation from the soil to the root hair and that there is no necessity for postulating the presence of a discrete intervening layer

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[2] This paper was written by the senior author and is based upon experiments formulated in conference by both authors. The experimental work was performed by the junior author.

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[5] Unpublished data.

of soil solution or the excretion of acids by the plant to account for acquisition of such ions by plants. We suggest that this type of exchange could also take place if the soil particles and root-hair surfaces carry mutually exchangeable anions. That such exchanges may occur, but to widely varying degrees, in practically all soils is extremely probable. Such exchanges could dominate in soils whose colloidal particles approach saturation for particular ions and are in contact with kinds of root surfaces which are likewise highly saturated with ions of appropriate sign of charge (+ or —). This latter condition might be met by some kinds of plant root surfaces and not by others; thus the superior acquisitive power of certain plants for certain ions (for example, rye for phosphate) might be accounted for.

The second type of causes of failure to obtain correlations between acid extractions and growth or absorption (on the part of the plant) is largely of a technical character.

Several causes are rather obvious. Thus, the larger *amounts* of acid solutions necessarily used in the laboratory treatments exceed the buffering capacity of the soil; they produce greater shifts in H ion concentration, and dissolve larger amounts of a given ion—for example, phosphate—than would be likely to dissolve in the field. Moreover, the relative amounts of a given ion extracted from different soils by acid vary with the amounts of acid used and with the magnitude of the shift in H ion in the equilibrium solutions produced by equal amounts of acid.

A good illustration of this is afforded by data previously reported from this laboratory (*8*):[6] Two soils of similar physical properties and approximately equal pH were treated with varied amounts of acid and the soils subjected to displacement in the manner usual in this laboratory (*1*). When the amount of acid was theoretically equivalent to 1/24 *N*, the first soil shifted the pH of the displaced solution to 5.9, the second to 6.6. The amounts of phosphate brought into solution were 29.5 mg and 5.0 mg respectively, a ratio of 6 to 1.

In a comparative experiment with acid applications equivalent to 4/24 *N*, the first soil shifted the pH of the displaced solution to 4.5, the second to 6.2; and the amounts of PO_4 were 67.6 mg and 6.0 mg respectively, a ratio of 11 to 1.

Again, using the same data, if the results are studied on the basis of the shift in pH instead of amount of added acid, at pH 6.5 the amounts of PO_4 brought into solution were 11.0 mg and 6.0 mg, respectively, or a ratio of about 2 to 1. At pH 5.5, the amounts of PO_4 were 55.0 mg and 3.0 mg respectively (an actual decline from that at pH 6.5), or a ratio

[6] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

of 18 to 1. Thus, if the figures are used to show the relative differences in supplying power of the two soils, the relation would be:

$$\begin{cases} 6 \text{ to } 1 \text{ for small amounts of acid} \\ 11 \text{ to } 1 \text{ for larger amounts of acid} \end{cases}$$

or

$$\begin{cases} 2 \text{ to } 1 \text{ for one increment of shift in pH} \\ 18 \text{ to } 1 \text{ for another increment of shift in pH} \end{cases}$$

These discrepancies are due to the fact that the acid comes in contact with different kinds of particles, and ions released by one kind of particle or compound become subject to secondary reactions. In the field, on the other hand, individual root hairs may influence solution from individual particles and the plant can remove dissolved substances before they have opportunity to react with other types of soil particles, or compounds, or with the free ions in the soil solution. This is also well illustrated by the work referred to above. Thus, it was shown that one of the effects of increasing acidity in a series of equilibrium treatments is to dissolve calcium or other ions, which in turn precipitate phosphate. Thus the phosphate figures so obtained do not reflect the magnitude of the effect of acid produced by H ion at the interphase boundary between a single particle and a root hair.

The effects of the type of secondary reactions (chemical and mass-action effects) just referred to, are now generally recognized and must, of course, be taken into account in interpreting data derived from the acid treatment of soil. It would, however, be unsafe and even absurd to conclude that this type of secondary reaction (precipitation) is the exclusive cause of the removal of phosphate from acid extracts. Many workers[7] have observed that various soil and colloidal clay minerals have great capacity to remove PO_4 from solutions and have ascribed this to an adsorption. Some of the data reported are, unfortunately, subject to the criticism that release of Ca ion by exchange with H ion could have caused the precipitation of phosphate and thus vitiated the conclusion that the effect is an adsorption phenomenon.

A notable attempt to elucidate the rôle of adsorption in phosphate fixation was that of Russell and Prescott (*7*). These investigators were unfortunate in that their experiments were performed at a time when the importance of the secondary chemical reactions discussed above were not generally recognized. Moreover, their conclusions were largely based on adsorption isotherms conforming to the Freundlich equation. Comber (*2*) pointed out the possibility of chemical reactions, and Fisher (*3*) showed the inadequacy of conformity to the Freundlich equation

[7] See the bibliography in Murphy (*5*).

as the exclusive test of an adsorption. These criticisms of the conclusions from the particular experiments are, of course, valid, but they do not prove that adsorption may not have played a part in determining the actual experimental results. Whether complexes capable of adsorbing phosphate were present in substantial amounts in these particular soils is perhaps of no general interest at this late date. The point is that an overemphasis of the rôle of secondary chemical reactions, important as these are in many soil types, has prejudiced the interpretation of data from experiments with phosphate in which the secondary reactions may have been caused by adsorption or exchange.

Murphy (*5*), in the accompanying paper, shows that a certain soil (Aiken clay loam), in which the colloidal clay component was largely of the kaolinitic type (as shown by X-ray diffraction photographs), reacted under experiment in the same manner as would have been expected from the studies of finely ground kaolinite carried on concurrently. In experiments with kaolinite, the material manifested the usual properties of colloidal alumino-silicates characterized by a low silica : alumina ratio. The kaolinite removed cations (K) most effectively from alkaline solutions and anions (PO_4) from acid solutions. The absence of ions capable of precipitating PO_4 precludes any other explanation than that of an adsorption. The similar behavior of the soil definitely points to its natural kaolinite component and to an adsorption reaction as the cause of the negligible amounts of phosphate removed from the soil by acid. Crops planted on the soil manifest all the symptoms of phosphate deficiency and the soil is practically immune to phosphate fertilization except when the applications are beyond economic possibility or where the fertilizer is localized in immediate contact with the absorbing roots. Phosphate adsorbed by this soil (and by the kaolinite) is not brought into solution except to a negligible extent by acid until the amount of acid is sufficient to shift the reaction of the equilibrium solution to about pH 1.0 when the aluminum silicates are breaking down, as demonstrated by the copious solution of Al and the release of soluble and colloidal SiO_2.

The paper in which these results are reported (*5*) also demonstrates a substantial phosphate-adsorbing capacity for other types of clay minerals (Volclay and bentonite). The much greater adsorbing capacity of kaolinite depends on its being finely ground. Even when finely ground, however, the kaolinite particles are probably much coarser than the particles of Volclay and bentonite as shown by the physical properties of liquid mixtures, ease of filtration, etc. The clays with high silica : alumina ratio are highly dispersed in water and are probably in a very fine state of subdivision in most soils. The kaolinite in natural soils (*6*) appears to

vary more in particle size than other clay minerals, and phosphate-adsorbing capacity of kaolinitic soils must therefore vary widely, even between soils of equal kaolinite content.

It is obviously impossible to devise a technique yielding index figures that can be applied with exactness to express the acid solubility of phosphate from the *individual* phosphate-carrying particles of the soil. With the vast majority of arable soils, a variable proportion of the phosphate rendered labile (dissolved) by acid is precipitated or adsorbed. Fairly good correlations between acid-soluble phosphate of soils and the phosphate-fertilizer requirement of plants are sometimes claimed. Such correlations must, however, be limited to slightly buffered soils, containing only small amounts of adsorbing clay minerals or clay minerals of low specific adsorbing capacity or clay minerals which are nearly saturated with phosphate.

The preceding discussion of data on or conclusions from acid extractions of soils concerned equilibrium extractions with "strong" acid (HCl). Many suppose, however, that the use of "weak" acids remedies some of the defects pointed out above.

The use of buffered acids, of course, prevents the rise of H ion concentration in the equilibrium solution and eliminates the secondary chemical precipitations, which tend to lower the phosphate figures actually obtained by use of unbuffered acid. In the field, however, the H ion concentration about an individual phosphate-carrying particle would rise greatly or little, as a result of CO_2 excretion by the plant, according to the distance of the particles of potentially soluble neutralizers, such as $CaCO_3$ or Ca-clays, from the phosphate-carrying particle. Thus, if the neutralizing power of a given soil is primarily due to $CaCO_3$, the amount of phosphate in solution at any one time in the field, as a result of acid excretion by plants, would depend upon the size and distribution of the $CaCO_3$ particles. In the laboratory at equilibrium, however, the phosphate dissolved by acid would be primarily a function of the total amount of $CaCO_3$.

Perhaps the most unfortunate occurrence in the history of soil-phosphate investigation was the choice of citric acid as a solvent. Dilute solutions of citric acid *do not dissolve* phosphate from the adsorption complex of soils: the citrate anion *displaces* phosphate from the adsorption complex.

But the plant cannot do this unless it excretes citrate ion, OH ion, or some other ion which is highly adsorbed by the clay complex, or by hydrogels when these are involved. Possibly some plants excrete enough organic acids containing anions which are strongly adsorbed. This spe-

cial ability could account for a relatively greater power of such plants to acquire phosphate from a given adsorption complex. Even if some plants could be shown to do this to a considerable degree, even if the contact phenomenon emphasized by Jenny is accepted *in toto,* it is certain that plants in general acquire adsorbed phosphate with great difficulty unless the individual particles are highly saturated (*9*) and the number of such particles is great.

We hesitate to use the term "availability," but in the sense in which this term is commonly applied, the phosphate contained in clay minerals is only slightly available,[8] while that removed by *dissolving* phosphates is extremely available, unless prevented from dissolving by a high buffer power on the part of the soil.

Thus, two soils extracted with citric acid could give equal and relatively large amounts of phosphate (as compared with the great majority of soils), and yet be very different in phosphate-fertilizer requirements. If in one soil, the phosphate in the citric-acid extract were actually derived from solution of phosphate, probably no fertilizer would be required. If, however, the phosphate in the acid extract were derived from an adsorption complex, a need for fertilization would almost certainly be indicated for most plants.

A simple experiment reveals all the facts necessary to the above conclusion. Finely ground kaolinite was partially saturated with phosphate. Five-gram portions, containing 61.45 mg PO_4, were brought to equilibrium with the respective solutions as noted. The following amounts of PO_4 were removed at equilibrium (1:5 suspensions), out of a possible 61.45 mg:

	pH value	PO_4 removed, mg
0.1 *N* Citric acid	2.27	21.88
0.005 *N* Hydrochloric acid	2.28	1.00
0.1 *N* Hydrochloric acid	1.01	6.84
0.1 *N* Sodium hydroxide	12.00	50.00

Hydrochloric acid (0.1 *N*) dissolves Al from kaolinite and releases small amounts of phosphate. Phosphate is *not displaced* from the adsorbed condition by hydrochloric acid of any pH short of one which breaks down the alumino-silicates of the adsorbing complex. In the pH range possible about an individual particle of clay complex in normal soils, there is no release of adsorbed phosphate as a result of a specific "acid" effect. Under such circumstances, phosphate is released only if the acid is one with a strongly adsorbed anion.

[8] Unless the particles are highly saturated and the *amounts* of phosphate so held are large.

FACTORS AFFECTING PHOSPHATE AVAILABILITY

From the preceding analysis, we suggest that the points to be determined in laboratory studies of soils in order to predict the need for phosphate fertilizers are as follows:

1. The amounts of phosphate liberated by acids of a type whose anion is not adsorbed by clay minerals or Fe hydrogels.[9]
2. The shift in pH caused by added acid, because this indicates whether increased acidity in the field would or would not be likely to shift the pH enough to cause the solution of phosphate to the extent observed in the laboratory.
3. The degree of phosphate saturation of the adsorbing clay minerals or adsorbing hydrogels.
4. The total phosphate (fusion analysis of the soil).

We now present for analysis the results of a series of experiments with a single soil, carried out in such a manner as to differentiate between precipitation and adsorption effects. All data represent 1:5 equilibrium extracts of 100 grams of a fine sandy loam soil (known as No. 117 in this laboratory) with amounts of acid (or alkali) producing the pH values noted at equilibrium. Series of extracts were made on the soil alone, soil + excess $CaCO_3$, soil + 400 mg Ca as $CaCl_2$, soil + 1 gram finely ground kaolinite.[10] The soil was chosen as one having no kaolinite and having little buffering power according to standards accepted for soils of this region. The soil does contain other clay minerals, and undoubtedly some of its phosphate is held as adsorbed phosphate. Moreover, the exchange complex does release calcium on acid treatment but not nearly so much as highly buffered soils of this region. When such a soil is extracted with dilute HCl, the resulting figures represent the amount of phosphate derived from solution of individual phosphate-carrying particles minus the phosphate precipitated or adsorbed.

The points on the curve representing the acid side of neutrality (see curve *A*, fig. 1) are obviously lower than they would have been if the phosphate-carrying particles could have been isolated and these alone extracted. This cannot be done, and calculations of the magnitude of this depressive effect from calcium released or amount of clay present are of little value because of the complexity of the equilibrium solution and lack of knowledge of the amounts of each clay mineral and the specific adsorbing capacity of each such mineral in this particular soil.

The curve (curve *A*, fig. 1) can, however, be analyzed and the dominating factors which determine it characterized. If the data are plotted in

[9] Possibly Al hydrogels in some soils at certain pH values.

[10] Data not presented for soil + excess $CaCO_3$. All acid treatments with $CaCO_3$ in excess, gave about equal pH values and equal phosphate figures.

absolute terms (curve *A*, fig. 2), the curve is practically a straight line on the acid side in the H ion range between pH 5.17 and 3.65. The slight change in the slope of the line at the lower pH values can be accounted for either by a small amount of precipitation or by adsorption effects or by both. The straightness of the line, however, indicates that the major effect is solution of phosphate-carrying particles or compounds. On the alkaline side (curve *B*, fig. 2), the curve is apparently logarithmic and

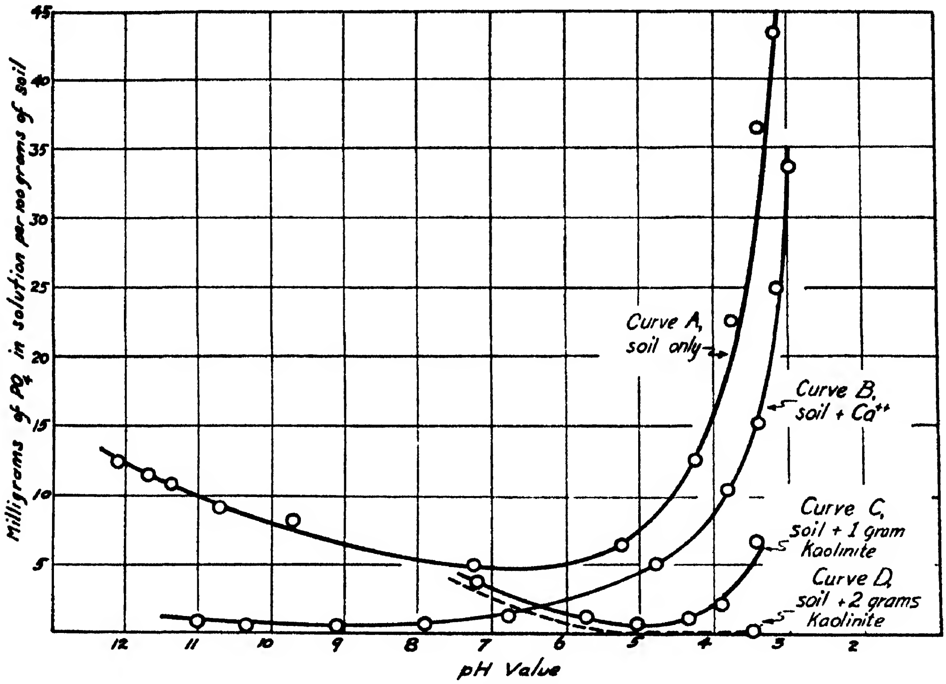

Fig. 1.—Phosphate determined in 1:5 equilibrium extracts from a variously treated slightly buffered soil, by acid and by alkali. Concentrations of HCl and NaOH used were 0.002, 0.004, 0.006, 0.008, and 0.010 *N*. The data for the kaolinite treatment on the alkaline side were essentially the same as for the untreated soil and are omitted to avoid confusion of the lines.

can be shown (if plotted logarithmically) to obey the Freundlich equation. All of the common phosphate compounds, which do not hydrolyze, become less and less soluble with increasing alkalinity. The rise observable with increasing alkalinity could be due to hydrolysis of iron, aluminum, or manganese phosphates (*8*); or to exchange of OH with PO_4 adsorbed either by clay minerals or by iron or aluminum hydrogels (*5*). As a matter of fact, an exchange of OH for the PO_4 of the clay minerals is undoubtedly the cause of the increase in this particular soil, but it really does not matter because all three classes (clay minerals, hydrogels,

and iron or aluminum phosphate) of phosphate complex would act in the same manner with OH.

As may be seen from curve *B* of figure 1, addition of 400 mg Ca ion as $CaCl_2$ to 100 grams of soil depressed the solubility of phosphate throughout the entire range. On the alkaline side, the PO_4 released from the soil minerals by OH exchange is almost completely precipitated by a very

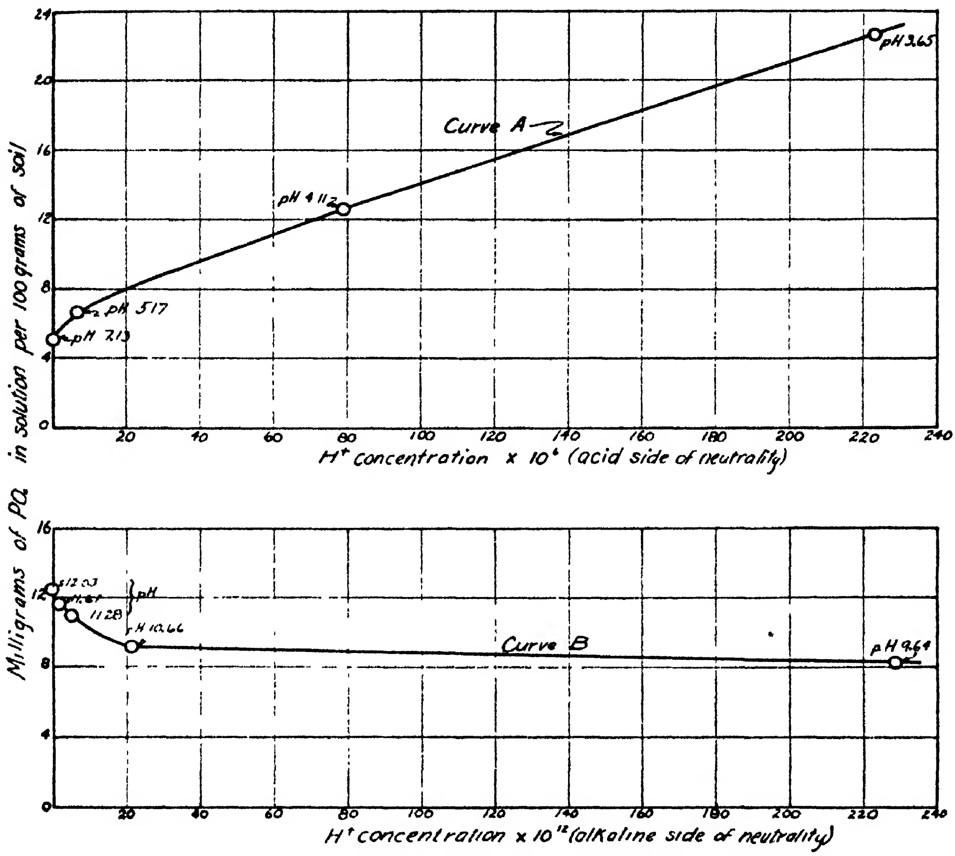

FIG. 2.—Data from curve *A* of figure 1 presented in absolute terms.

small application of Ca ion. In the soil alone (curve *A*, fig. 1) there was not enough dissolved calcium (that is, Ca ion) at pH values above 7 to produce this effect; the phosphate released from the soil minerals therefore remained in solution as determined.

As may be seen from curve *C*, figure 1, 1 gram of kaolinite in 100 grams of soil removes a large proportion of the PO_4 released by acid, the adsorption increasing with increasing H ion (decreasing pH) at first, but later rising. This latter rise is evidently due to the fact that, at the lower pH values, the clay mineral is approaching saturation. That this is so is

shown (curve *D*, fig. 1) by the supplementary experiment: 2 grams of kaolinite removes all of the phosphate released by acid. *Evidently then, the degree of saturation and the amount* of adsorbing clay minerals in a soil is what determines their ability to conceal the true acid solubility of soil phosphate. Moreover, the degree of saturation of the clay mineral particles and the number of those particles in immediate contact with potentially acid-soluble phosphate particles must determine the amount of phosphate that is actually dissolved in the soil by biologically produced acids. If the clay minerals are only slightly saturated with phosphate and very numerous as compared with other phosphate-carrying particles, the excretion of acids by the plant will merely move the phosphate from the acid-soluble particles to the practically insoluble adsorption complex. If the plant gets phosphate from this complex, it does so, not by acid solution, but as a result of exchanges, as suggested by Jenny for cations. Here again, the amount of phosphate the plant can get from the adsorption complex is determined by degree of saturation and amount of adsorbed phosphate.

An interesting point is whether any factor other than pH in the complex mixture of ions in the acid extracts has any effect upon the adsorbing capacity of the clay minerals. At pH 3.6, the amount of phosphate released by the soil was 23.5 mg (curve *A*, fig. 1). The amount of phosphate *not* removed by the kaolinite was 3.4 mg (curve *C*). The kaolinite had, therefore, adsorbed 20.1 mg and was in equilibrium with a solution containing 3.4 mg. We, accordingly, made up a number of solutions, all containing 23.5 mg of PO_4 (from KH_2PO_4) in 500 cc, after adjusting the H ion concentration to give values of about pH 3.6 when 500 cc of solution was brought to equilibrium with 1 gram of kaolinite. One of these equilibrium mixtures, with a pH of 3.69, had approximately the same PO_4 *ion* content as the soil-kaolin-acid mixture, as shown below:

Equilibrium mixture	pH	Phosphate adsorbed, mg	Phosphate in solution, mg	Total phosphate, mg
100 grams soil + 1 gram kaolinite + 500 cc dilute HCl....	3.60	20.1	3.4	23.5
1 gram kaolinite + 500 cc dilute KH_2PO_4 solution..............	3.69	19.3	4.2	23.5

The distribution of phosphate between kaolinite and solution is essentially the same in the two mixtures, which are otherwise very different. Apparently the only factors which affect the phosphate-adsorbing capacity of the clay minerals are PO_4 concentration and pH.

INFORMATION REQUIRED TO DIAGNOSE PHOSPHATE DEFICIENCY

In the preceding discussion, the points emphasized are the importance of determining the degree of saturation of the adsorbing complexes (minerals, hydrogels, etc.); the specific capacity of potentially soluble phosphate particles to dissolve in acid; the effect of buffers (Ca ion, etc.) in lowering the apparent solubility of these particles; and the total phosphate content of the soils. How are these factors to be determined, and how can they be evaluated?

The degree of saturation of the adsorbing constituents of the soil can be readily calculated if the amount of adsorbed phosphate actually present in the soil is known, together with the amount of phosphate the adsorbing minerals *could* hold at saturation, that is, the adsorption capacity. These figures can be ascertained from 0.1 *N* NaOH equilibrium extracts[11] of the soil itself and of the soil *after* saturation with PO_4. The actual concentration of the NaOH is not important provided it is uniform for both treatments, is sufficiently concentrated to overcome buffering on the alkaline side of neutrality, and produces a high pH in the equilibrium solution.

The adsorption capacity of an adsorption complex is, of course, affected by the pH at which the adsorption takes place (and the concentration of phosphate in the treating solution), but if concentrated solutions of KH_2PO_4 at pH 4.5 are used in the proportion of 500 cc to 100 grams of soil, the pH of the equilibrium solution will usually lie between 4.5 and 7.0 and the absolute amounts adsorbed will not vary greatly. Moreover, in those soils in which adsorption effects are important, the adsorbing capacity for PO_4 is very great as compared with the amounts actually in the adsorbed condition; so that a considerable variation in the determined adsorption capacity could not materially affect the calculated values of what we call the "degree of saturation," or the "adsorption deficit."

The specific capacity of phosphate-carrying particles to dissolve in acids cannot be determined exactly except in soils having no buffer capacity and which are also free from adsorbing complexes. The general order of magnitude of this factor can, however, be inferred from the amounts of phosphate extracted by acid and the determined buffer capacity of the soil. Five volumes of 0.01 *N* HCl to 1 gram of soil will shift the pH more than any plant could do, but not enough to overcome the buffering

[11] Equilibrium extracts do not give the entire amount of adsorbed phosphate, but they give almost all, and all that need be considered in these relations.

power of soils. The shift in pH actually observed by this treatment gives an appropriate measure of the buffer capacity of the soil.

The determination of total phosphate by fusion analysis presents no difficulty. This datum is necessary to give weight to the quantity factor, in supplementing conclusions drawn from the data showing the degree of saturation.

APPLICATION OF CHEMICAL DATA TO PROGNOSIS OF PHYSIOLOGICAL PHOSPHATE DEFICIENCY

The evaluation of the criteria suggested will obviously depend upon comparisons between the known physiological responses to phosphate fertilization of individual soils, and the figures or indexes obtained from such soils by the methods outlined.

This we believe we have accomplished for a group of fourteen soils of widely different characters. The minor defects of our correlations are apparently due to the incompleteness of the physiological data and to variation in the specific capacity of different plants to acquire phosphate from particles of equal degree of saturation. The first defect can be overcome by obtaining further evidence of physiological deficiency in the particular soils. The second defect can never be entirely eliminated, but its elimination is not necessary if the data are not interpreted too narrowly. Fertilizers are not so valuable that the most effective dosage must be predicted with a high degree of accuracy. To be able to say that for the generality of crops such and such a soil will probably respond to phosphate applications, is sufficient.

Many of the soils used in these experiments are the same as those used by Hibbard (*4*), who gives additional data on them.

Phosphate-deficient Soils.—By referring to table 1, the reasons why the soils manifesting great phosphate deficiency are unable to supply the plant are perfectly obvious. Soils 103, 69A, and 64 yield practically no acid-soluble phosphate: their unsatisfied adsorption capacity is so great (adsorption indexes, 3.28, 1.54, and 0.93) as compared with their phosphate content, and the degree of saturation is so small that the adsorption complex holds phosphate with great avidity, and the plant cannot easily remove it.

Soil 78 can yield practically no acid-soluble phosphate; this is definitely referable to its high adsorption index. The degree of saturation is high and individual particles of adsorption complex could be expected to yield phosphate well to the plant, but the low total phosphate, the effect of which is reflected in adsorption index, prevents plants from getting phosphate rapidly.

TABLE 1

CHEMICAL DATA (PO_4) FROM SOILS OF KNOWN PHYSIOLOGICAL RESPONSE

Soil No. and description	PO_4 per 100 grams of soil: Adsorption capacity*	PO_4 per 100 grams of soil: In adsorbed condition†	Present degree of saturation $\left(\frac{\text{col. 1}}{\text{col. 2}}\right)$	PO_4 per 100 grams of soil: Adsorption deficit (col. 1 − col. 2)	PO_4 per 100 grams of soil: Total‡	Adsorption index $\left(\frac{\text{col. 4}}{\text{col. 5}}\right)$	PO_4 per 100 grams soil, acid-soluble§	pH of acid equilibrium	Mineral characteristics¶	Buffering ability	Phosphate deficiency indicated by plant growth
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
	mg	*mg*	*per cent*	*mg*	*mg*		*mg*	*pH*			
103. Aikin clay	547	22.0	4	525	160	3.28	0.2	4.78	Kaolinite	Moderate	Extreme
69A. Sites clay loam	290	44.0	15	246	160	1.54	0.8	3.89	Kaolinite	Moderate	Great: extreme for tomatoes, etc., but not for fruit trees
78. Fine sandy loam	105	30.4	29	75	45	1.67	1.2	2.74	Kaolinite and montmorillonite	Slight	Great: extreme for tomatoes; moderate for barley; none for fruit trees
64. Vina silt loam	174	16.4	9	158	170	0.93	0.6	4.81	Kaolinite	Moderate	Great: marked for tomatoes; moderate for alfalfa
38. Vina silty clay loam	181	23.6	13	157	250	0.63	4.8	5.22	Kaolinite	Substantial	Intermediate: none for some time
36. Farwell sandy loam	126	13.6	11	112	250	0.45	1.9	6.19	Kaolinite and montmorillonite	Substantial	Intermediate: marked for tomatoes
40. Altamont-Olympic-loam wash	172	41.6	24	130	330	0.39	13.4	4.86	Mineral X and montmorillonite	Moderate	None
37. Nord sandy loam	51	10.4	20	40	240	0.17	5.5	8.50	Faintly kaolinite	Extreme	Intermediate: moderate for tomatoes, much less than No. 36 at first
1C. Yolo silty clay loam	55	26.6	48	28	200	0.14	5.1	7.09	Montmorillonite	Substantial	None
68A. Tujunga fine sand	23	4.4	19	19	140	0.14	17.9	3.50		Slight	Unknown, no information
53. Delhi sand	29	18.0	62	11	80	0.14	46.7	2.86		Slight	None for first crop; later marked for tomatoes
30. Fresno fine sandy loam	32	6.0	19	26	160	0.16	40.6	3.00		Slight	None for some time; after several crops, moderate for tomatoes
75. Yolo loam	87	12.8	15	74	232	0.32	9.1	6.25	Montmorillonite	Substantial	None
80. Hanford fine sandy loam	8	4.4	55	4	115	0.04	0.6	7.75	Mineral X	Extreme	Great: very marked for tomatoes; largely corrected by making soil acid

* The adsorption capacity represents the phosphate removed by 0.1 *N* NaOH (1:5, 1-hour equilibrium suspensions); from soil subjected for 15 hours to a KH_2PO_4 solution (1:5) containing approximately 3 mg of PO_4 per cc and having a pH of 4.5. The free KH_2PO_4 present in the soil was leached out with distilled water before applying the NaOH treatment.

† Direct determination on soil—0.1 *N* NaOH (1:5) 1-hour equilibrium syspension.

‡ Fusion analyses.

§ Determined from a 24-hour equilibrium suspension of soil and 0.01 *N* HCl (1.5).

¶ Determined from X-ray photographs.

Soil 80 has a negligible adsorption deficit and negligible amounts of adsorbed phosphate. Plants suffer because the potentially acid-soluble phosphate is prevented from coming into solution as a result of the high buffer capacity of the soil.

Soils of Intermediate Phosphate-supplying Power.—Of this group, soil 36 shows early physiological deficiency. Soils 37 and 38 show deficiency in subsequent crops. Soil 36 yields very little acid-soluble phosphate, primarily owing to its substantial buffering capacity, aided by a substantial capacity to adsorb phosphate (adsorption capacity 126, adsorption index 0.45). In soils 37 and 38, the amounts of acid-soluble phosphate are greater than for No. 36 but are not large, owing entirely to buffering in soil 37 and primarily to adsorption in soil 38. The buffering of No. 37 is capable of being remedied by (acidic) applications in the field, but the acid-soluble phosphate of No. 38 should decline under cropping, as indicated by the high adsorption index (0.63).

Soils of Good Present Supplying Power.—The lack of present physiological deficiency for plants in soils 40, 53, and 30 is easily accounted for by the comparatively large figures for acid-soluble phosphate. The relatively lower acid-soluble figure of No. 40 as compared with Nos. 53 and 30 is referable to the higher adsorption index (0.39 as against 0.14 and 0.16); inasmuch as the soil is only moderately buffered.

Soils 30 and 53 both manifest phosphate deficiency after a relatively small number of crops have been grown. This cannot be explained on the basis of a transfer of potentially acid-soluble phosphates to the adsorption complex, for the adsorption indexes are low; it must be otherwise accounted for. This we believe can be done as follows: The very high figures for acid solubility (*in vitro*) reflect a high acid solubility for both the soils. The buffering power of both of these soils is negligible, so that their individual phosphate-carrying particles could not be prevented from delivering PO_4 to the soil solution with normal CO_2 excretion by plants. This means a concentrated soil solution, with respect to phosphate,[12] and a "luxury" adsorption of phosphate by the plant. The total phosphate in soil No. 53 is very low and the total phosphate in No. 30 is subaverage. The total phosphate in both soils should decline rapidly because of luxury consumption by the plant and cause an absolute deficiency in a very few years.

Soils 1C and 75 give sufficient acid-soluble phosphate to account for a reasonably good supplying power in spite of substantial buffering. Since the soils are buffered, the adsorption complex could play little part in

[12] Soil No. 30 had originally the highest phosphate concentration of displaced solution of all encountered in this laboratory.

lowering the acid-soluble phosphate actually accessible to the plant even if the adsorption deficits were much higher than they are in both cases.

The physiological efficiency of soil No. 68A is unknown, but the comparatively high acid-soluble figures and low buffering capacity with the negligible adsorption capacity (adsorption index 0.14) indicates that the soil cannot be physiologically deficient at the present time.

We refrain from further analysis of these data because we realize that the limited number of soils and the incompleteness of our data with respect to the physiological supplying power of the soils could lead to erroneous conclusions as to the significance of specific figures obtained in the laboratory. We hope to present further evidence at a later date from larger numbers of soils, the physiological response of which is determined with three types of plants—plants with great acquisitive power for adsorbed phosphate, plants with slight acquisitive power, and plants with what may be called "average acquisitive power."

CONCLUSIONS

The phosphate-carrying particles of natural soils may be divided into two sharply defined classes—those which dissolve in acids (that is, both ions enter solution) and those which do not dissolve in acid, but which either hydrolyze in alkaline solutions or release phosphate only as a result of anion exchange. The phosphate accessible to plants (derived from acid-soluble particles) depends upon the PO_4-ion concentration of the soil solution[13] to a minor degree, and upon the amounts of PO_4 brought into solution by H ion at the interphase boundary between the root hair and the individual phosphate-carrying particle. The phosphate accessible to plants (derived from hydrolysis or from adsorbed phosphate) depends upon the degree of saturation of the hydrogels or adsorbing clay minerals and the amount of phosphate so held.

The phosphate brought into solution from the individual soil particles by acid cannot be exactly determined, but can be inferred from acid extractions, supplemented by a knowledge of the buffer capacity of the soil.

The amounts of phosphate on the hydrogels and clay minerals can be determined by extracting the soil with alkali. The degree of saturation of particles holding phosphate in this manner can be determined by comparing amounts of PO_4 extracted by alkali from the soil before and after saturation with phosphate.

We believe it possible to obtain limiting figures from chemical data, easily obtainable in the laboratory, which will indicate degrees of physio-

[13] As defined in this laboratory, that is, ions free to move in the water of the soil independently of solid phase or colloidal particles.

logical deficiencies in soils. We suggest certain preliminary and tentative figures and magnitudes, subject to change as a result of further studies of a larger group of soils and for plants of different sensitivities: If acid-extractable phosphate is high[14] (9 mg or more per 100 grams of soil), most plants will have no present difficulty in obtaining phosphate from the soil. If acid-extractable phosphate is low (about 1.0 mg or less), plants will usually acquire phosphate with difficulty, but the difficulty will be greatly enhanced if the buffer capacity is low and the adsorptive index is high. If the acid-extractable phosphate is intermediate, plants will probably have difficulty in acquiring phosphate when the buffer capacity is extremely high (compare soil 37 and soil 1C); or even in the absence of extreme buffering capacity, when the adsorption index is high.

The effect of a high adsorptive power (high adsorption index) in preventing a plant from acquiring potentially acid-soluble phosphate in the field is directly associated with the buffering power of the soil: (*a*) if the adsorption index is high and the buffering capacity is low, the conditions are highly adverse; (*b*) if the buffer capacity is high, the adsorption index is of no import in this connection.

The ability of a soil having a high adsorption index to deliver PO_4 from the adsorption complex to the plant in the field may be substantial for certain plants, provided that the degree of saturation and the number of particles carrying adsorbed phosphate is great, and if such plants can give off anions (for example, citrate) capable of being adsorbed. That plants with superior acquisitive power for phosphate have this power for the reason indicated is highly probable, particularly if the amount of contacts (root hair to soil particle) are great as a result of an extensive root development on the part of the plants.

Until further studies by the methods outlined have been performed with soils of very high organic-matter content, and on soils whose buffering capacity (or alkalinity) is due to sodium instead of calcium, we prefer to exclude such soils from our interpretation. We have data in hand which indicate that certain of our methods are applicable to peat soils, but the interpretations we have ventured above may have to be modified in some respects for this type of soil material.

SUMMARY

The causes of failure to obtain correlations between plant growth and the acid-extractable phosphate of soils are partly inherent and partly technical.

The rôles of adsorption complexes, hydrogels, and acid buffers, as

[14] By our technique.

affecting the analytical figures obtained by acid extraction of soils, are outlined.

The specific "acidity effect" of reagents cannot be measured in soils containing substantial amounts of adsorbing colloids if the anion of the reagent is itself adsorbed by the soil.

The kinds of chemical determination necessary in the prognosis of phosphate deficiency are announced.

A critical analysis of the quantitive effects of Ca ion and kaolinite in determining the phosphate solubility of a type soil is presented.

Hydrogen-ion concentration, phosphate concentration, present degree of saturation, and adsorption capacity of the adsorbing complex determine the removal of acid-dissolved phosphate both *in vitro* and in the field.

Chemical data on fourteen soils are analyzed in the light of these findings to illustrate how the present physiological supplying power of the soil could be deduced from the data.

Tentative standards represented by figures and magnitudes are suggested as a basis for predicting the current phosphate-supplying power of soils.

ACKNOWLEDGMENT

We are especially indebted to P. L. Hibbard of this laboratory, whose extensive studies of the acid solubility of soil phosphate have indicated the desirability of using dilute acid and the importance of interpreting results of acid extraction with reference to the buffering capacity of soils.

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THE RÔLE OF KAOLINITE IN PHOSPHATE FIXATION

H. F. MURPHY

CONTENTS

THE RÔLE OF KAOLINITE IN PHOSPHATE FIXATION[1, 2]

H. F. MURPHY[3]

WHEN A SOLUBLE PHOSPHATE is brought in contact with the soil, reactions take place which remove a great deal, if not all, of the phosphate from solution. This phenomenon has been called "the fixation of phosphates by soils." Such a term carries no implication of the means by which removal takes place or of the product formed; it conveys only the idea that the phosphate has been changed and is present in some form that is only slightly soluble under the prevailing conditions. To the scientist who is interested in soil relations, plant relations, and the complex soil-plant system, more than the mere fact that the "phosphate is fixed by the soil" is required. The mechanism of the fixation is important to the soil specialist, while the means of resupplying the soil solution or the plant from the phosphate so held is important to the plant physiologist.

The investigations reported here were undertaken to obtain more fundamental data on the manner of phosphate fixation in soils not controlled by the calcium system.

PHOSPHATE-FIXATION SYSTEMS

The reactions converting phosphates into less soluble forms are several in number. The various types of fixation may now be classified into a few groups, which will be discussed in the following paragraphs.

Group 1, Chemical Precipitation.—Until rather recently, chemical precipitation has been considered the primary cause of phosphate fixation in all soils. This precipitation has been attributed largely to Ca, Fe, Al, Mg, and Mn.

A great many soils are governed by what may be termed the "calcium system." Soils with such a system predominate in arid and semiarid regions and also occur in the more humid sections. In these soils, calcium is the predominating cation, although leaching may have removed it to a considerable degree. In some instances, leaching has been so severe that the only calcium left is found in a few local zones, and the soils are very acid. Such soils cannot be considered strictly within this group and will

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[1] Received for publication June 22, 1938.

[2] Submitted in partial fulfillment of the requirement for the Degree of Doctor of Philosophy in the University of California.

[3] Graduate student of the University of California 1930–31, and 1937–38; now Associate Professor of Soils, Oklahoma Agricultural and Mechanical College.

not be dealt with here. Where calcium is the controlling cation, it reacts with soluble phosphates and withdraws the phosphate ions from solution. Calcium phosphates, however, are appreciably soluble in an acid medium; therefore, the production or introduction of a hydrogen-ion producing substance tends to redissolve the calcium phosphates formed.

Teakle (*46*)[4] has shown that calcium phosphates are relatively insoluble on the alkaline side of neutrality, and in the presence of an excess of calcium, they become insoluble at about pH 6. This means that to liberate phosphate for plant use in such a system hydrogen ions must be produced in an amount sufficient to lower the pH at the absorbing surface below this value. Slightly acid soils belonging to this system and having an appreciable amount of calcium in the replaceable form are able to supply the plant with phosphate without difficulty, provided the potential supply is adequate. Gaarder and Nielsen (*15*) have shown the behavior of the phosphate ion in the presence of free ions of iron, aluminum, calcium, sodium, and magnesium.

In acid soils, the fixation of phosphates has been considered as due to the precipitation of $FePO_4$ and $AlPO_4$. The probability is against such a simple fixation, because free Fe and Al ions are not present in the soil solution to any appreciable extent except in very acid soils. That $FePO_4$ and $AlPO_4$ may be partially precipitated from an artificial system at pH ranges common to acid soils is ably shown by several investigators (*46, 9*), but this does not prove that Fe or Al ions are present in sufficient quantity to fix applied soluble phosphates rapidly. Moreover, certain experiments (*32*) have indicated the accessibility of such phosphates to plants at pH values of 6 and 7. If $FePO_4$ and $AlPO_4$ were the products of fixation, it would seem that soils having a fair supply of phosphate and within this range of soil reaction should be able to supply the plant with the necessary phosphorus for growth. Truog (*48*) found that precipitated $FePO_4$ and $AlPO_4$ produced, with a few exceptions, good growth.

Group 2, Mutual Precipitation of Phosphates by Iron, Aluminum, and Silica Hydrogels.—Evidences of the adsorption type of reaction have accumulated during the last few years. Russell and Prescott (*42*) in 1916 ascribed some of their results to adsorption phenomena, but the criticism of their work by Fisher (*11*) and by Comber (*5*) discredited the idea for a time. In 1921 Harrison and Das (*20*) concluded that in noncalcareous soils the retention of P_2O_5 is mainly due to adsorption, whereas in calcareous soils retention by adsorption is either nonexistent or entirely masked by other causes. Shortly thereafter, the work of Gordon and his

[4] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

associates (*19, 25, 45*) and of Mattson (*27–30*) began to appear in the literature in support of a colloidal mechanism. Other recent investigations (*6, 18, 31, 33, 34, 35, 37, 38*) furnish confirming evidence of this kind of action.

The simpler iron and aluminum compounds found in the soil are oxides or hydrous oxides. While iron and aluminum ions may be present, the amount of these is appreciable only when the soil becomes very acid. The ordinary soil solution is very low in these ions as well as in phosphate ions. These facts lend support to the idea that the hydrated oxides of these materials, because of their colloidal nature, are responsible for considerable adsorption of phosphate ions. Heck (*21*) considers the active iron and aluminum in soils as existing in the forms of hydrated oxides.

Artificially prepared hydrogels and hydrosols of iron and aluminum have been shown (*16, 25, 45, 47*) to remove PO_4 from solution. Gordon and his associates (*25, 51*) found that while iron and aluminum hydrogels were able to hold on to the adsorbed phosphate tenaciously against distilled water and certain sulfates, $0.1N$ solutions of NaOH and NH_4OH were capable of removing some of the adsorbed phosphate, and the plants were able to utilize PO_4 from the freshly prepared material. Ellett and Hill (*10*) stated in 1917 that "if the yield is taken as a measure of availability, iron and aluminum do not fix phosphoric acid in forms unavailable to the wheat plant." In their experiments, they used iron and aluminum hydroxides as fixing agents for monocalcium phosphate and superphosphate (acid phosphate). Tiulin (*47*) prepared an iron gel of varying degrees of saturation of phosphate and showed that plants were able to secure phosphorus from it in accordance with the degree of saturation. Ford (*12, 13*) and Dean (*7*) present evidence showing the formation of complex phosphate addition compounds to certain hydrated iron and aluminum oxides. Mattson (*29*) and Pugh (*35*) have demonstrated that the phosphate content of the iron or aluminum hydrogel complex is variable; it increases as pH is lowered and decreases as the pH is raised.

Group 3, Adsorption of Phosphates by Alumino-Silicates.—Mattson (*30*) shows that artificially prepared alumino-silicates as well as some naturally occurring colloids containing silica are capable of adsorbing phosphates, and that the PO_4 content of the complex varies indirectly with pH. Pugh and du Toit (*36*) report that silicates and phosphates may be regarded as substituted hydroxides, and that the OH ion replaces SiO_3 and PO_4 ions in exactly equivalent proportions in synthesized ferric silicates and phosphates.

Roszmann (*40*) suggests the possibility that the clay complex fixes

phosphate. Bradfield (*2*) suggests that phosphates may in part be retained on the surfaces of the colloidal alumino-silicates in the soil, and that other anions may replace it. Scarseth (*44*) found that a prepared bentonite retained phosphate, and suggested that the adsorption on the colloidal surfaces at pH 5.5 to 6.1 was due to the aluminum valence. "Ferriferated" bentonite increased the phosphate retained. The PO_4 ion was found to be replaceable by OH and SiO_4 anions.

Gilbert (*17*) reported that normal phosphate fertilization of some Rhode Island soils during a period of thirty-five years had not reduced the power of phosphate adsorption significantly, irrespective of the phosphate carrier used. A high rate of liming reduced the adsorption power. Furthermore, phosphate was fixed by these soils from buffer solutions of monopotassium phosphate adjusted to pH 5, 6, and 7; but was given up by the soil when the pH was lowered to 2 or was raised above 10. Truog (*48*) showed that freshly precipitated iron and aluminum phosphates were utilizable by plants; but that after the first crops they were less so, and suggested that these older basic phosphates may have combined with acid silicates to form very resistant insoluble compounds.

GENERAL EXPERIMENTAL METHODS

The methods employed were varied according to the nature of the individual experiments and are described briefly in connection with these experiments. A few methods were common to many experiments, however, and these are described at this point to avoid repetition. The phosphates were determined volumetrically by the usual ammonium molybdate procedure. Potassium was determined volumetrically by titrating the precipitated potassium cobalti-nitrite with standard $KMnO_4$. The pH values were determined by the glass electrode except in the greenhouse experiments, where the hydrogen electrode was employed. Where shaking was employed, it was generally done on rollers which turned the containers slowly along their horizontal axes. Exceptions to this shaking procedure will be noted.

INFLUENCE OF IRON ON PHOSPHATE FIXATION

Iron Hydrosols at Different pH Values in the Presence of an Excess of Hydrosol.—In experiment 1a, a solution of $FeCl_3$ was made of such concentration that when the other reagents were added, 500 cc contained 340 mg of Fe. The iron was precipitated as hydroxide at various pH values by the use of different amounts of dilute NH_4OH. A solution of KH_2PO_4 sufficient to furnish 175.6 mg of PO_4 was then added to make a total volume of 500 cc in each case. The suspensions were shaken by

hand, and after standing for about 1 day, the filtrates were tested for PO_4. The results are given in table 1.

TABLE 1

THE RETENTION OF PHOSPHATE IN THE PRESENCE OF AN EXCESS OF IRON HYDROSOL

Color of filtrate	Reaction		PO_4 fixed	
	Suspension	Filtrate	Amount	Per cent*
	pH	*pH*	*mg*	*per cent*
Reddish amber	2 13	2 14	15.60	8 88
Clear amber	2 23	2 26	85 60	48 74
Very slightly yellow	2 53	2.64	173 35	98.72
Water-clear	2.82	3 11	173 85	98 98
Water-clear	4 94	5 09	170 35	97 01
Water-clear	6 50	6 50	129 60	73 80
Water-clear	7 36	7 32	110 60	62 98
Water-clear	8 12	8 17	94 60	54 44

* PO_4 fixed as a percentage of that added.

The amount of iron (340 mg) was arbitrarily chosen, but represents the amount removed from 100 grams of Aiken clay loam in citric acid (1:5) equilibrium extract.[5] The exact procedure was to digest 10 grams of soil with 100 cc of *N* citric acid on a steam bath for ½ hour. The PO_4 used was approximately equal to the total PO_4 content of 100 grams of this soil.

TABLE 2

THE INFLUENCE OF pH ON $FePO_4$ PRECIPITATION IN THE PRESENCE OF AN EXCESS OF IRON

Color of liquid	Reaction		PO_4 fixed	
	Suspension	Filtrate	Amount	Per cent*
	pH	*pH*	*mg*	*per cent*
Amber	2 10	2 10	105 00	60 00
Amber	2 27	2 27	150 60	85 76
Slight amber	2 51	2 56	165 60	94 30
Slight yellow tinge	2 79	2 92	170 35	97 01
Water-clear	3 17	3 47	174 35	99 29
Water-clear	5 87	6 12	171 85	97 86
Water-clear	7 33	7 30	175 35	99 86
Water-clear	7.80	7 82	174 56	99.41
Water-clear	8 24	8 27	173.10	98 57

* PO_4 fixed as a percentage of that added.

In experiments 1a and 1b, the hydrosol was formed before adding the phosphate solution, whereas in experiments 2a and 2b the phosphate solution was added to the iron solution before adding the NH_4OH. This

[5] Hagan, R. M. Physiological deficiency in soils as related to mechanism of phosphate release. Unpublished manuscript. 1937.

has a considerable bearing on the retention of PO_4, as will be recognized in comparing the graphs in figures 1 and 2. Figure 1, curve *a*, is a graphic representation of the data in table 1.

In experiment 1b, the same procedure as in experiment 1a was used except that the amount of $FeCl_3$ was only in slight excess of that neces-

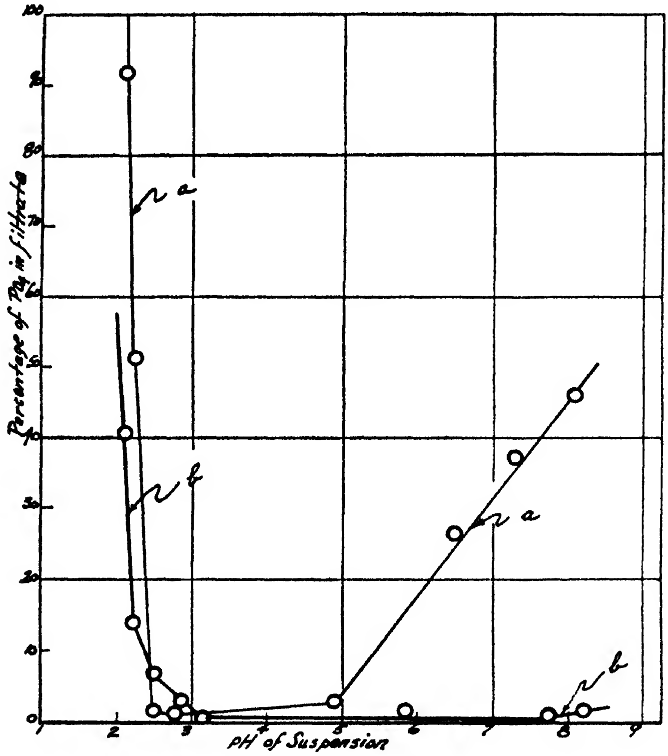

Fig. 1.—Curve *a*, the influence of pH on the adsorption of phosphate by an iron hydrosol containing a large excess of Fe over PO_4; curve *b*, the influence of pH on the precipitation of phosphate in the presence of a large excess of Fe.

sary to combine with PO_4 to give $FePO_4$. The results are graphically represented in figure 2, curve *a*.

Iron Phosphates at Different pH Values in the Presence of an Excess of Iron.—In experiment 2a, a solution of $FeCl_3$ was added to a KH_2PO_4 solution, and the pH was changed by adding various amounts of dilute NH_4OH. The total volume was 500 cc and contained 340 mg of Fe and 175.6 mg of PO_4. The shaking and period of standing were the same as in experiment 1a. The results are recorded in table 2 and are shown graphically in figure 1, curve *b*.

In experiment 2b, the same procedure as in experiment 2a was used except that the amount of $FeCl_3$ employed was in only slight excess of

that necessary to combine with the PO_4 to give $FePO_4$. The results are shown graphically in figure 2, curve *b*.

Discussion of Experiments with Iron.—The data indicate that even in the presence of an excess of iron hydrosol an appreciable amount of PO_4 is left in solution at pH values of 6 and above. Hence, if hydrous iron

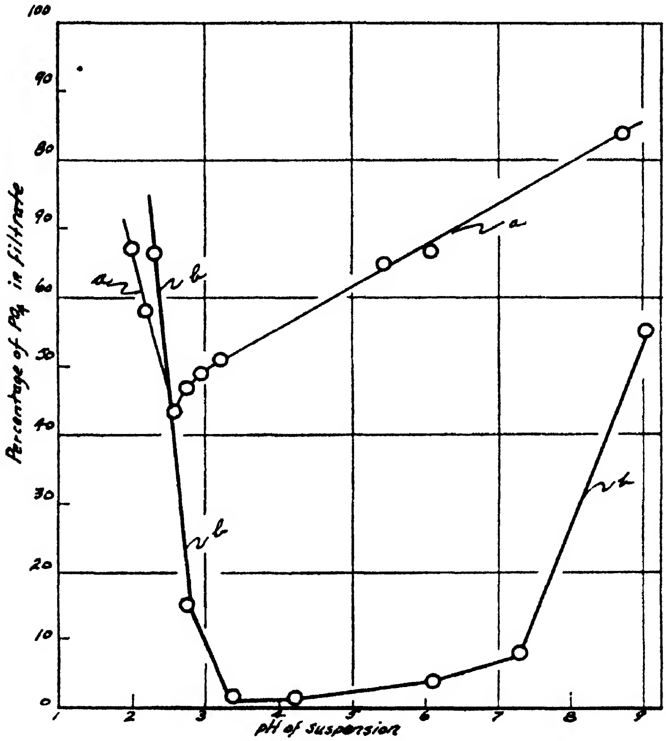

Fig. 2.—Curve *a*, the influence of pH on the adsorption of phosphate by an iron hydrosol containing only a slight excess of Fe over PO_4; curve *b*, the influence of pH on the precipitation of phosphate in the presence of a slight excess of Fe.

oxides are functioning as the adsorbing system in a given soil, at pH values of about 6 there should be considerable PO_4 in the soil solution available for plants, especially after applying a fertilizer containing soluble phosphate.

Not much iron is found in the soil solution until a high acidity is reached; therefore, the fixation as $FePO_4$ (the *b* curves in figs. 1 and 2) has a very limited effective range and could not account for rapid fixation in soils having only a slight acidity. Fixation by iron in such a pH range would be largely limited to the hydrous oxides present. Free nonhydrated iron oxide (hematite) has been found (*13*) unable to fix phosphate.

STUDIES ON A SOIL HAVING A HIGH FIXING POWER

Certain soils have greater capacity to remove phosphates from solution than others. Colloids from such soils are characterized by having a low silica : sesquioxide ratio. The Aiken soil series is representative of this group and has been chosen for the studies involved.

Composition.—The composition of Aiken clay loam and its colloid was determined by the methods of the Association of Official Agricultural Chemists (*1*). The colloid was secured by placing 1 kg of soil in a 5-gallon earthenware jar, agitating thoroughly each day, and removing the top 8 cm of suspension every 24 hours. This was carried out over a 30-day period. The suspension was filtered with Pasteur-Chamberlain filter tubes using suction. The chemical analyses of soil and colloid are given below:

Constituent	Soil, per cent	Colloid, per cent
SiO_2	38.63	30.98
Fe_2O_3	12.42	12.40
Al_2O_3	34.43	39.90
CaO	1.68	1.68
MgO	1.20	1.05
PO_4	0.18	0.22
Ignition loss	15.41	17.40

The ratios were as follows:

$SiO_2 : Al_2O_3$	1.90 : 1	1.32 : 1
$Fe_2O_3 : Al_2O_3$	0.23 : 1	0.20 : 1
$SiO_2 : R_2O_3$	1.54 : 1	1.10 : 1

The percentage phosphate content of the colloid is higher than that of the soil. This is in accordance with data of other investigators (*3*, *12*) who have made a study of the distribution of the chemical constituents in the various soil separates. It indicates that the finer fractions of the soil have a higher capacity for combining with soluble phosphates.

The Release of Phosphate by Acid Treatment.—Tests were made with HCl at various strengths on untreated Aiken clay loam. The ratio of soil to solution in each case was 1 :5.

Such tests, if the acidity is great enough to dissolve appreciable quantities of iron and aluminum. should liberate increasing amounts of PO_4 if the PO_4 already present in the soil is in the form of iron or aluminum phosphate. Particularly is this the case if the pH is below 2.5, as is shown by figures 1 and 2. The results (table 3) did not indicate any increase in the PO_4 content of the filtrate with increase in HCl from equilibrium mixtures shaken for 1 hour. Evidently such minerals are absent; or if

present, their presence is obscured by a secondary reaction involving clay minerals of a type discussed in a later section.

The Recovery of Phosphate from Superphosphate Treatments with Dilute NaOH.—Four percolators were filled with air-dried Aiken clay loam. The cross-sectional soil area at the top of the percolator was equivalent to 1/500,000 acre. The soil was moistened to field capacity with distilled water, and superphosphate of various-sized particles was placed

TABLE 3

PO_4 LIBERATED FROM AIKEN CLAY LOAM BY DIFFERENT HCl SOLUTIONS

Approximate normality of acid	Reaction of suspension	Fe in solution*	PO_4 in filtrate per 100 grams of soil
N	*pH*		*mg*
0 100	1.66	+++	<0 2
0 075	1 68	+++	<0 2
0 050	2 16	++	<0 2
0 025	2 93	++	<0 2
0 010	4 16	Trace	<0 2
0 002	5 64	None	<0 2
0 001	5 83	None	<0 2

* As shown by NH_4OH precipitation.

on top of the moistened soil surface of each percolator at the rate of 600 pounds per acre. The soils were leached with distilled water until 2,000 cc of leachate was secured from each percolator. This required approximately 2 days. The samples were allowed to stand in the percolators and air-dry for one week. They were then divided into layers. A 1:5 suspension of each of the various soil layers in 0.1*N* NaOH was made. The samples were shaken 1 hour, filtered, and the PO_4 content of the filtrate was determined.

Sodium hydroxide was chosen as a medium because numerous experiments (*50* and unpublished data[6]) have shown that low figures are secured from its use if the PO_4 in the soil is controlled by a calcium equilibrium system; furthermore, high figures are secured where the phosphate is in the form of iron or aluminum phosphate or is held by adsorption. Hydrolysis of iron and aluminum phosphates occurs as the pH is increased above pH 7 (*15, 26, 46*).

The results are reported in table 4.

The Recovery of Phosphate from Superphosphate Treatments by Acid Equilibrium Systems.—Previous experiments,[7] as well as those performed by the writer (table 3), show that ordinary acid equilibrium

[6] Burd, J. S., unpublished data.

[7] Hagan, R. M. Physiological deficiency in soils as related to mechanism of phosphate release. Unpublished manuscript. 1937.

extractions of this soil give practically no PO_4 in the filtrate. However, according to table 4, more of the added phosphate remained in the soil as a calcium phosphate when larger rather than when small particles of

TABLE 4
THE INFLUENCE OF PARTICLE SIZE ON THE RECOVERY OF PO_4 FROM SUPERPHOSPHATE BY $0.1N$ NaOH

Treatment	Depth	Weight of soil fraction	Total PO_4 soluble in $0.1N$ NaOH	PO_4 not recovered*
	inches	*grams*	*mg*	*mg*
10- to 20-mesh† superphosphate, 156.8 mg PO_4	0.0–0.5	109.7	99.15	
	0.5–1.0	117.9	41.18	
	1.0–2.0	130.1	38.12	
	2.0–3.0	96.0	19.20	
	3.0–4.0	88.0	22.88	
	4.0–5.0	91.0	19.11	
	Total	632.7	239.64	56.99
Untreated‡		632.7	139.83	
60- to 100-mesh superphosphate; 167.2 mg PO_4	0.0–0.5	115.5	103.56	
	0.5–1.0	111.9	42.03	
	1.0–2.0	144.8	48.88	
	2.0–3.0	101.0	20.20	
	3.0–4.0	73.0	17.52	
	4.0–5.0	99.0	22.77	
	Total	645.2	254.96	54.83
Untreated‡		645.2	142.59	
Finer than 200-mesh superphosphate; 153.3 mg PO_4	0.0–0.5	81.4	81.33	
	0.5–1.0	107.8	40.92	
	1.0–2.0	148.4	63.54	
	2.0–3.0	103.0	28.84	
	3.0–4.0	102.0	24.48	
	4.0–5.0	95.0	27.55	
	Total	637.6	266.66	27.55
Untreated‡		637.6	140.91	

* This is calculated from the amount of PO_4 added in the superphosphate, the amount of PO_4 $0.1N$ NaOH extracted from untreated soil, and the amount of PO_4 the NaOH actually extracted from the treated soil.

† This means that the superphosphate passed through a 10-mesh sieve, but was retained on a 20-mesh sieve.

‡ $0.1N$ NaOH removed 22.10 mg of PO_4 from 100 grams of untreated soil.

superphosphate were used; these results indicate that perhaps an acid extraction would give a further indication of this amount.

Accordingly, the equivalent of 20 grams of moisture-free soil from the 0.0–0.5-inch layer of each treatment was shaken for 1 hour with HCl so as to furnish a 1:5 filtrate with a pH of 3. The results are shown in table 5.

The experiment is inconclusive, since the acid may have dissolved the residual calcium phosphate; and this may have been immediately adsorbed by the soil colloids.

The Recovery of Phosphate from Superphosphate Treatments by Acid Leaching.—The purpose of acid leaching was to determine if, during the HCl equilibrium experiment, any phosphate was dissolved which was subsequently fixed.

Three 100-gram samples of Aiken clay loam were weighed out, one was

TABLE 5

HCl EQUILIBRIUM AND HCl LEACHING STUDIES OF AIKEN CLAY LOAM AFTER TREATING WITH SUPERPHOSPHATE

Fineness of superphosphate used	HCl equilibrium studies*		HCl leaching—PO_4 in filtrate per 100 grams of soil†
	Reaction of suspension	PO_4 in filtrate per 100 grams of soil	
	pH	*mg*	*mg*
10- to 20-mesh	3 07	0 2	29 77
60- to 100-mesh	3 07	0 2	
Finer than 200 mesh	2 99	0 2	15 04
None	2 93	0 2	Trace

* 20 grams of soil from 0.0-0 5-inch layer of treatment in table 4 used in this experiment.

† 560 mg of superphosphate per 100 grams of soil used.

treated with 560 mg of 10- to 20-mesh superphosphate, one was treated with 560 mg of superphosphate finer than 200-mesh, and no phosphate was added to the third.

The superphosphate was thoroughly mixed with the soil. The samples were placed in pint jars and 50 cc of distilled water were added. The jars were loosely closed to prevent evaporation and were kept at room temperature for 8 days. At the end of this time, the soil was removed from each jar and spread out in a thin layer over 15-cm filter papers in Buechner funnels. Each sample was leached rapidly with 500 cc of 0.1N HCl, filtering being hastened by suction. The time required was 5 minutes. Samples of the filtrates were tested for their phosphate content. The results are tabulated in table 5.

Phosphate-Fixation Capacity.—Samples of 100 grams of Aiken clay loam were shaken for 1 hour with 500 cc of water and enough monocalcium phosphate to furnish the amounts of PO_4 shown in table 6. The samples were filtered, and the PO_4 content of the filtrates was determined.

The residues were re-treated with the same quantities of monocalcium phosphate as in the first treatment, and the rest of the procedure repeated. In table 6 are also recorded the amounts of PO_4 fixed by this second treatment, and the combined fixation for both treatments.

The Effect of Grinding.—A 75-gram charge of the Aiken clay loam was ground in a ball mill for 6 days. Its ability to fix phosphate was determined along with that of unground soil, Aiken colloid, and kaolinite that had been ball-milled for 9 days.

The procedure followed was to weigh out 1-gram samples into small bottles; add 50 cc of KH_2PO_4 solution (pH 4.48) containing a total of 355.24 mg of PO_4, and shake for 100 hours. After the shaking process, the suspensions were allowed to stand for 1 day to permit the settling out of the solid materials. The PO_4 content of the filtrates was then determined. The results were as follows:

Substance	pH of filtrate	PO_4 fixed per 100 grams of soil, mg
Aiken clay loam	5.22	2,372
Aiken clay loam, ball-milled	5.89	6,232
Aiken colloid	5.51	3,858
Kaolinite, ball-milled, 9 days	6.07	10,988

Discussion of Aiken-Clay-Loam Investigations.—The analyses (p. 350) indicate that the soil chosen for the study has a low silica : sesquioxide ratio, and that the total iron content is much the same in the colloidal and noncolloidal fractions.

If all the iron in the soil were active and free to combine with PO_4, 100 grams of the soil would be able to fix approximately 14.7 grams of PO_4. If only the iron in the clay fraction is active (calculation would indicate approximately 50 per cent of the soil is colloidal matter) the 100 grams of soil would be able to fix about 7.3 grams of PO_4.

The 100 grams of soil (table 6), after two exposures to a high concentration of monocalcium phosphate, retained approximately 2.5 grams of PO_4. This would require that between 25 and 30 per cent of the iron in the clay fraction be active and free to combine. If the soil had been treated with more phosphate, more PO_4, would probably have been fixed.

Unpublished data[8] indicate that normal citric acid removes approximately one-third of a gram of iron from 100 grams of this soil. Assuming that this is all the iron that is free to react with PO_4, it could combine with only about 0.6 grams of PO_4. The soil actually retained more than four times this amount (table 6). The pH of normal citric acid is 1.82, which is much below pH 4.26, the pH of the filtrate after fixation from the high concentration of monocalcium phosphate; and that much acidity should bring into solution, if not all, at least most, of the iron which is free to react with the phosphate to form $FePO_4$ or the iron phosphate complexes.

[8] Hagan, R. M. Physiological deficiency in soils as related to mechanism of phosphate release. Unpublished manuscript. 1937.

The active iron that could be instrumental in the fixation process must be of a hydrous oxide form and have sufficient surface exposure. In view of the amount dissolved by citric acid, iron of this character could scarcely be present in sufficient amount.

After the soil is ground in the ball mill, its ability to fix phosphate increases very greatly, which would indicate other factors are entering into the problem of phosphate fixation in this soil.

The fixation capacity is extremely high, and therefore saturating the soil with phosphate would apparently be impractical. When a heavy application is made, however, some phosphate is left for plant use.

TABLE 6

PHOSPHATE FIXATION BY AIKEN CLAY LOAM WITH TWO MONOCALCIUM PHOSPHATE TREATMENTS

Sample No.	PO_4 added (each treatment)	PO_4 in filtrate	PO_4 fixed per 100 grams of soil					
			First treatment		Second treatment		Total	
	mg	*mg*	*mg*	*per cent*	*mg*	*per cent*	*mg*	*per cent*
1	25 26	0 35	24 29	96.16	23 61	93 47	47 90	94 81
2	125 91	2 55	123 36	97 97	109 00	86 57	232 36	92 27
3	624 85	152 61	472 24	75 58	275 41	44 08	747 65	59 83
4	3,125 76	1,693 93	1,431 83	45 81	1,036 65	33 16	2,468 48	39 49

The low figures secured with the acid treatments on the fertilized soil (table 5) indicate a high degree of fixation and precludes the presence of much calcium phosphate. These data, however, do not tell where the fixation took place in the soil column.

Since NaOH extracts give large figures for iron, aluminum, and colloidally formed phosphates, they can be used to determine in which soil layers the fixation occurred. The data in table 4 show that extreme fixation took place in the surface ½ inch of soil and in no case was there any appreciable penetration of the applied phosphate below a depth of 2 inches.

The larger particles of fertilizer appear to have some advantage over the finer particles in retaining their phosphate as calcium phosphate in the soil. This is indicated by the acid-leaching data given in table 6. Because of the limited leaching, these figures cannot be taken as the total amount of calcium phosphate present, but they represent a high percentage of it.

A concentrated HCl digestion of the coarse particles which were removed from the acid-leached soil showed that they contained a total of 21 mg of PO_4. Hence, the coarse particles after a week's contact with moist soil retained less than one-third of their original phosphate content.

INVESTIGATIONS WITH KAOLINITE

The failure to secure an increase in released PO_4 (table 3, p. 351) when the soil was extracted with HCl at pH 2 and lower (the reaction at which phosphated iron hydrosol and iron phosphate are both soluble) lead to an investigation of the type of clay present in the soil, and its capacity to remove phosphate from solutions. X-ray investigations showed that the clay was kaolinic in character (fig. 3, *B*). Data presented on page 350 show it to have a low silica : sesquioxide ratio.

TABLE 7
THE RETENTION OF PHOSPHATE BY CRUSHED KAOLINITE
(Suspension* shaken 1 hour)

Test No.	Weight of kaolinite	PO_4 added	PO_4 fixed per 100 grams of kaolinite
	grams	*mg*	*mg*
1	25	23 33	25 0
2	20	17.57	23 7
3*	20	8.79	22 5
Average			23 7

* 1.0:5.0 suspension for tests 1 and 2, 1.0:2.5 suspension for test 3.

Retention of Phosphate by Crushed Kaolinite.—Since the clay was so identified, samples of crushed kaolinite were tested for their ability to fix phosphate from a 1 :5 suspension of dilute monocalcium phosphate. The shaking was for a 1-hour period.

The results are recorded in table 7.

The rather coarse kaolinite used fixed PO_4 at the rate of 23.7 mg per 100 grams. This establishes a rather high fixing power even for fairly coarse kaolinite, and immediately suggests a probable source of fixation for soils (containing kaolinite) whose fixing capacity cannot be assigned to precipitation by calcium or to mutual precipitation with iron or aluminum hydrosols.

The Effect of Grinding.—In an experiment to determine the effect of fineness of kaolinite upon the quantity of PO_4 fixed, a sample of 125 grams of kaolinite was placed in a ball mill for 6 days. At the end of this time all but 50 grams was removed. The 50-gram charge was left in the mill, and at intervals 1-gram samples were removed. The ability of the various samples ground for different periods of time to fix phosphate was determined. In making this determination, 1-gram samples were placed in small bottles with 50 cc of KH_2PO_4 solution containing 6.8 mg of PO_4 per cc and having a pH of 4.47. The suspensions were shaken on a roller

for 100 hours. They were removed and allowed to settle for 1 day, when the phosphate content of the supernatant liquid was determined.

The results are recorded in table 8. Grinding increased immensely the capacity of the kaolinite to retain phosphate. Since the kaolinite in the soil is in a finely divided condition, it must have a great capacity to fix phosphate.

The Effect of Reaction as Indicated by Leaching.—A solution of KH_2PO_4 was modified by the addition of NaOH and water so as to give

Fig. 3.—X-ray photographs: *A*, of the kaolinite used in the experiments; *B*, of the Aiken colloid; *C*, of a referee sample of kaolinite.

solutions having pH values of 4.54, 6.23, 6.43, and 7.90. Each of these solutions had the same K and PO_4 content (1.1 and 2.8 mg per cc, respectively), but the sodium content varied according to the amount of NaOH necessary to change the pH. Kaolinite, which had been ground in a ball mill for 5 days in a 150-gram charge and then 2 days in a 75-gram charge,

TABLE 8

THE EFFECT OF GRINDING KAOLINITE ON PHOSPHATE FIXATION
(1:50 suspension with KH_2PO_4 solution per 100 cc; shaken 100 hours)

Size of charge	Cumulative grinding time	pH of filtrate	PO_4 fixed per 100 grams of kaolinite
grams	*days*	*pH*	*mg*
	Storeroom sample	4 59	822
125	6	5 43	4,752
50	7	5 63	6,332
49	8	5 69	8,114
48	12	5 72	9,104
47	15	5 78	10,292

was weighed out in four 10-gram lots, placed in beakers; and 100 cc of solution was added, a different solution being placed in each beaker. The beakers and their contents were warmed in a water bath at 70° C for 3 hours. The suspensions were filtered with suction. The residue was then washed with three additional portions (15 cc, 50 cc, and 25 cc) of the

same phosphate solution as had been used earlier. The PO_4 content of the solutions less that in the filtrates was considered as the phosphate fixed by the kaolinite. The results are given in table 9.

The Effect of Reaction as Indicated by Equilibrium Study with PO_4 Constant.—One-gram samples of kaolinite, which had been ground in a ball mill for 7 days in a 75-gram charge, were suspended in 50-cc portions of solutions of KH_2PO_4 with different pH values. This amount of phos-

TABLE 9

THE FIXATION OF PHOSPHATE AT VARIOUS REACTIONS BY KAOLINITE AND ITS INFLUENCE ON THE REACTION OF THE FILTRATE

Reaction		PO_4 fixed per 100 grams of kaolinite	K fixed per 100 grams of kaolinite
Original solution	Filtrate		
pH	*pH*	*mg*	*mg*
4 54	6.20	1,956 7	342.5
6 23	6 40	1,243.3	491 7 (?)
6 43	6 40	863 8	409 3
7 90	7 24	274 9	452 9

phate solution contained 98.55 mg of PO_4. The pH was adjusted by the addition of KOH. The suspensions were shaken for 6 hours, and after standing 1 day, the supernatant liquid was analyzed.

The results are summarized in table 10.

The Effect of Reaction as Indicated by Equilibrium Study with Both K and PO_4 Constant.—Solutions of H_3PO_4, KOH, and KCl were mixed and made to volume to give solutions with different pH values but with a constant PO_4 and K content: 50 cc contained 183.8 mg of K (except where otherwise noted) and 160.7 mg of PO_4. One-gram samples of kaolinite ground as in the preceding experiment were suspended in 50 cc of each solution. The suspensions were shaken for 20 hours, then centrifuged, and the supernatant liquid analyzed immediately. The results are given in table 11.

The Effect of Concentration on Phosphate Fixation.—The effect of the concentration of KH_2PO_4 on phosphate fixation was studied in connection with different amounts of kaolinite which had been ground in a ball mill for 7 days. The total volume of solution was 25 cc in all cases. The kaolinite was weighed out in series of 1.0-gram, 2.5-gram, and 5.0-gram samples and one sample of each weight was added to each KH_2PO_4 solution. The suspensions were shaken for 12 hours, allowed to stand 7 days, filtered, and the filtrates were analyzed. The results are given in table 12.

The Effect of Heating.—According to several investigators (*24, 39*), kaolinite breaks down when heated to 450–500° C. This does not neces-

sarily mean that the new complex would not be able to fix phosphate, however.

Two 1-gram samples of kaolinite ground in a 150-gram charge for 5

TABLE 10

THE FIXATION OF PHOSPHATE BY KAOLINITE IN AN EQUILIBRIUM SYSTEM

Reaction		Content of solution*		Content of filtrate		Amount fixed per 100 grams of kaolinite*	
Original solution	Filtrate	K	PO_4	K	PO_4	K	PO_4
pH	*pH*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*
4.56	6 12	38 45	98.55	34 19	79 30	426	1,925
6.66	6 80	52 99	98 55	45 18	87 17	781	1,138
7.70	7 62	71 45	98 55	62 27	95 20	918	335

* A 50-cc portion of solution was shaken with 1 gram of kaolinite.

days in a ball mill were weighed out. One of these was heated to 500° C for 70 hours; the other was left unheated. Each was shaken with a solution of KH_2PO_4 (pH 4.60) containing 98.55 mg of PO_4 for 18 hours, and the amount of PO_4 fixed in each case was determined. There was no appreciable difference in the amount of PO_4 fixed, each fixing approximately 1,000 mg per 100 grams. The loss in weight during the heating period was 0.1345 gram. The pH of the filtrate after fixation in each case was 5.78.

TABLE 11

THE FIXATION OF PHOSPHATE FROM SOLUTIONS HAVING DIFFERENT pH VALUES, BUT WHERE THE K AND PO_4 CONTENTS WERE KEPT CONSTANT

Amount of K in solution	Reaction		Source of K	K fixed per 100 grams of kaolinite	PO_4 fixed per 100 grams of kaolinite
	Solution	Filtrate			
mg	*pH*	*pH*		*mg*	*mg*
183 8	1 87	3 15	None		13.776
	1 90	3 07	100 per cent KCl	501±	13.587
	5 41	5 91	60 per cent KCl, 40 per cent KOH	475±	3 068
	6 88	6 83	40 per cent KCl, 60 per cent KOH	423±	2,281
	10 09	8 03	20 per cent KCl, 80 per cent KOH	1,105	619
	12 24	10 51	100 per cent KOH	2,248	None
36 8	1 85	3 05	100 per cent KCl*	162	13.646
	2 31	5 15	100 per cent KOH*	529	9.529

* One-fifth the K content of the others.

In another case where 1 gram of kaolinite was heated at 450-550° C for 50 hours and subjected to shaking for 100 hours in contact with 50 cc of KH_2PO_4 solution (pH 4.43) containing 432.4 mg of PO_4, it fixed 53.6 mg of PO_4 as compared with 67.2 mg fixed by an unheated sample. The pH of the filtrate from the unheated sample was 5.87.

TABLE 12

THE EFFECT OF KH_2PO_4 CONCENTRATION ON PHOSPHATE RETENTION BY KAOLINITE

Reaction*			Content of original solution, per cc		Fixation per 100 grams of kaolinite						Ratio of millimols of PO_4 to milliequivalents of K fixed		
Original solution	Filtrate		K	PO_4	1.0-gram sample		2.5-gram sample		5.0-gram sample		1.0-gram sample	2.5-gram sample	5.0-gram sample
	2.5-gram sample	5.0-gram sample			K	PO_4	K	PO_4	K	PO_4			
pH	*pH*	*pH*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*			
4.21	6 30	6 49	10 580	26 344	6,905	18,861	4 411	13 604	2,617	9 629	*1.00*	*1.00*	*1.00*
4 28	Lost	6 39	6 348	15 806	4 737	13 200	2,505	7 274	1,924	5.486	1 12	1 27	1.51
4 32	6 14	6 36	4 232	10 538	†	8 732	1 662	4,876	1,282	4,061	1 15	1 19	1.17
4 38	6 25	6 54	2 116	5 269	†	3 956	1 050	2 634	780	2 315		1 21	1 30
												1 03	1.22

* Not determined on the filtrate from the 1 0-gram sample

† Not determined.

Heating to 500° C, which destroys the crystal structure as revealed by X-ray data, apparently has little effect in reducing the capacity of kaolinite to fix phosphate.

The Effect of Time.—Time (*6, 14, 22*) has been shown to be of considerable importance in phosphate fixation. This was studied by using two

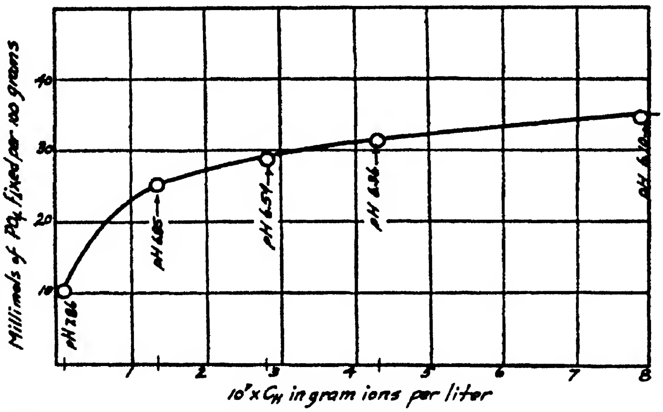

Fig. 4.—The relation between hydrogen-ion concentration of equilibrium solution and phosphate fixed. (Data taken from table 18.)

5-gram samples of finely ground kaolinite suspended in 50 cc of KH_2PO_4 solution. One of the suspensions was shaken for 4 hours and the other for 5 days.

The results are recorded in table 13. The data show very clearly that time is an element of importance in the fixation process, but even so, the fixation after a short exposure is of considerable magnitude.

TABLE 13

THE INFLUENCE OF TIME ON PHOSPHATE FIXATION BY KAOLINITE

Time of exposure	PO_4 in original solution*	PO_4 in filtrate	PO_4 fixed per 5 grams of kaolinite
	mg	*mg*	*mg*
4 hours	1,242 32	928 72	313 60
5 days	1,242 32	560 22	682 10

* Shaken with 5 grams of kaolinite.

The Effect of Degree of Saturation with Respect to PO_4 on Cation Exchange Capacity.—"Kaolinite phosphates" of different degrees of saturation were made by suspending ground kaolinite in solutions of KH_2PO_4 of different concentrations. After adsorption, the samples were filtered, and the residues were washed with distilled water and air-dried. The degree of phosphate saturation of the kaolinite samples was determined by analyzing the solutions before and after exposure. The base-

exchange capacity of the "kaolinite phosphates" was determined by the usual neutral normal ammonium acetate method used in the Plant Nutrition Laboratory. The results are shown in table 14 and in figure 5.

TABLE 14

THE BASE-EXCHANGE CAPACITY OF "KAOLINITE PHOSPHATES" OF DIFFERENT DEGREES OF PHOSPHATE SATURATION

Material		Exchange capacity		PO_4 fixed per 100 grams
Type	PO_4 per gram	Per 100 grams	Increase per 100 grams	
	mg	*milli-equivalents*	*milli-equivalents*	*millimols*
Kaolinite	0 0	26 85		
"Kaolinite phosphate"	14.55	31 92	5 07	15 31
"Kaolinite phosphate"	20 45	34 96	8 11	21.62
"Kaolinite phosphate"	36 37	41 05	14 20	36 29
"Kaolinite phosphate"	64 68	50.68	23 83	68 08

The Effect of the Oxalate Ion on the Adsorption of Phosphate by Kaolinite.—One-gram samples of ground kaolinite were treated with different ratios of 0.1*N* H_3PO_4 to 0.1*N* oxalic acid. The total volume in all cases was 50 cc. The suspensions were shaken for 6 hours and the phosphate and oxalate ions in the filtrates were determined. The oxalate ion

TABLE 15

THE FIXATION OF PHOSPHATE IN THE PRESENCE AND ABSENCE OF THE OXALATE ION

(1 gram of kaolinite used)

H_3PO_4	With oxalate ions present					With no oxalate ions	
	Amount of oxalic acid	Al in solution	Reaction of filtrate	PO_4 fixed per gram of kaolinite	C_2O_4 fixed	Reaction of filtrate	PO_4 fixed per gram of kaolinite
cc	*cc*		*pH*	*mg*	*mg*	*pH*	*mg*
50 0						3 42	134 32
37 5	12 5	++	3 28	72 35	?	3 79	105 66
25 0	25 0	+++	3 42	20 21	2 20	4 06	71 63
12 5	37 5	++++	3 13	0.28	None	4 80	34 99
0 0	50 0	+++++	2 52	—0 39	2 20		

in an H_2SO_4 solution was titrated against standard potassium permanganate. The aluminum content of the filtrates was qualitatively studied.

In another series no oxalic acid was added, but the same amounts of 0.1*N* H_3PO_4 were used as in the various mixtures with the volumes made up by adding distilled water. The filtrates from the latter series contained only a trace of aluminum. A comparison of the results is given in table 15.

In a study of KH_2PO_4 and oxalic acid systems at the same pH values, three different solutions were made to a pH of 4.8–4.9 as follows:

Solution 1.—50 cc of KH_2PO_4 solution + 60 cc distilled water. Solution 2.—50 cc of KH_2PO_4 solution + 50 cc 0.1*N* oxalic acid + 4.6 cc water + 5.4 cc *N* KOH. Solution 3.—50 cc of 0.1*N* oxalic acid + 54.8 cc water + 5.2 cc *N* KOH.

A 1-gram sample of kaolinite was suspended in 50 cc of each of these solutions. The suspensions were shaken for 4 hours and allowed to stand 12 hours before centrifuging. The filtrates were analyzed and the results tabulated (table 16). There was no aluminum present in any of the filtrates, as observed from ammonium hydroxide precipitation.

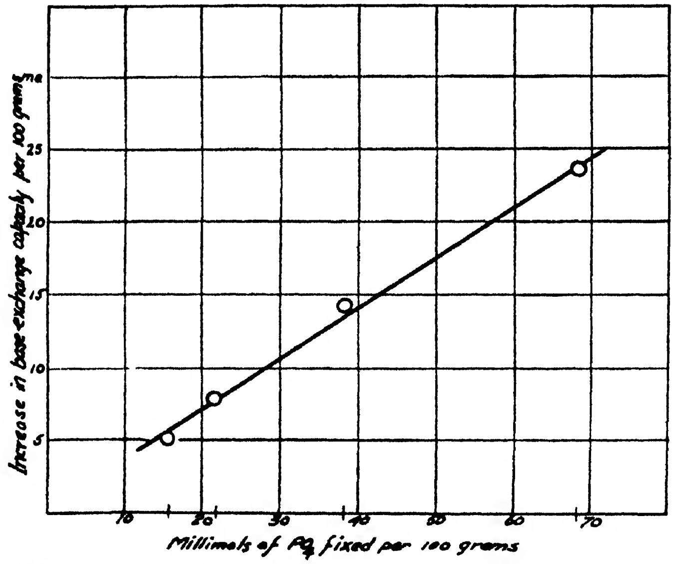

Fig. 5.—The relation of phosphate fixation by kaolinite to increase in base-exchange capacity.

The presence of the oxalate ion decreases the amount of phosphate fixed. In the previous experiment (table 15), this was probably due to the breakdown of the kaolinite (as evidenced by the aluminum in solution) because of the low pH of the initial solution. In the case of the experiment with KH_2PO_4 (table 16), there was no apparent decomposition of the kaolinite, yet there was much less phosphate fixed. The exact reason for the decrease in phosphate fixation is not apparent in this case.

Discussion of Kaolinite Investigations.—Kaolinite when finely ground (table 8) has great capacity to fix phosphate. The hydrogen-ion concentration is an important factor in the fixation process, but since the fixation is of great magnitude in the reaction range of agricultural soils, any soil possessing a kaolinic clay will exhibit this fixing power to a high degree. The fixation decreases as the OH-ion concentration increases

(tables 9, 10, 11, 13); at high pH values (table 11) the kaolinite complex is unable to adsorb phosphate. This is in opposition to cation fixation by kaolinite, which increases with increasing OH-ion concentration.

Since the magnitude of the fixation of phosphate is greatest in the acid range, the H_2PO_4 ion must play a considerable rôle. As indicated by figure 4 and data (table 17) prepared by Buehrer (*4*), as long as the hydrogen-ion concentration is sufficient to permit the presence of the H_2PO_4 ion, phosphate is fixed. At the higher pH values, the increased fixation of K (cation) lowers the pH of the filtrate. When the drop in pH

TABLE 16
THE FIXATION OF PHOSPHATE AND OXALATE IONS FROM SOLUTIONS HAVING AN INITIAL pH OF 4.8–4.9
(1 gram of kaolinite used)

PO_4 added	C_2O_4 added	Fixation per gram of kaolinite		
		PO_4	C_2O_4	K
mg	*mg*	*mg*	*mg*	*mg*
47 85	0 0	13 93		4 54
47.85	100 0	7 37	2 2	6 82
0 00	100 0		2 2	Not determined

(increase in hydrogen-ion concentration) is not sufficient to bring about the formation of the H_2PO_4 ion, the magnitude of phosphate fixation is small. Hence, the fixation is roughly proportional to the percentage of H_2PO_4 ions in the solution. The fixation of some phosphate in the pH range of 8 to 10 indicates that HPO_4 ions enter into the process to some extent.

The concentration of the phosphate solution (table 12) has an influence on the degree of phosphate saturation of the kaolinite. The higher the concentration the more nearly saturated will the kaolinite complex become. When a small amount of kaolinite is suspended in a concentrated phosphate solution, it fixes a relatively large amount of the soluble phosphate, while a large amount of kaolinite will fix practically all of the phosphate if the quantity of soluble phosphate is not extremely great. This has a practical application in the use of the phosphate fertilizers on soils having this type of clay.

As the kaolinite complex increases in phosphate content, its base-exchange capacity increases proportionally to the amount of phosphate fixed (table 14 and figure 5). Mattson (*27–30*) showed that phosphated sesquioxides possess a high cation-exchange capacity, and that certain soil colloids possessed this increased exchange capacity when they had adsorbed phosphate. This he attributed to an increase in the acidoid con-

tent of the complex through the adsorption of a polyvalent ion. The data in table 14 substantiate the results of Mattson. Since the data further indicate the importance of the H_2PO_4 ion and HPO_4 ion at the higher pH values, one of the possible reactions is as follows: $3\ CH_3COONH_4 + HO[Si(OH)_mAl]H_2PO_4 \rightarrow NH_4O[Si(OH)_mAl](NH_4)_2PO_4 + 3\ CH_3COOH$. The $(OH)_m$ designates OH groups which are nonreactive under the prevailing conditions.

When the pH of the extracting solution is somewhere below pH 1.5–2.0 and the ratio of solution to kaolinite is great (mass action), the kaolinite

TABLE 17

CONCENTRATION OF PHOSPHATE IONS IN SOLUTION AT VARIOUS HYDROGEN-ION CONCENTRATIONS BASED ON AN ASSUMED TOTAL CONCENTRATION OF 1 PART PER MILLION* OF PO_4

pH	H_3PO_4	$H_2PO_4^-$	$HPO_4^=$	$PO_4^{\equiv}$
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
3	0 084	0 92	†	†
4	0091	99	0 0019	
5	00089	98	0194	
6	0 00076	79	164	
7	†	33	66	
8		048	94	
9		0 00495	98	0 00035
10		†	0 99	0 0035

* From Buehrer (4)

† The blank spaces denote insignificant values for the purpose involved.

begins to break down, and as a result, its ability to fix phosphate decreases. When the pH approaches a value of 1, there is a complete loss of ability to fix phosphate. This effect was produced when a 1-gram sample of kaolinite was shaken for 10 hours with 50 cc of HCl of pH 1.2. The filtrate had a high aluminum content. The residue fixed very little phosphate, either from a solution of H_3PO_4 (pH 1.9), or from a solution of KH_2PO_4 (pH 4.3). Before treating with the HCl, a sample of the original kaolinite fixed between 140 and 160 mg of PO_4 per gram. Apparently a breakdown of the kaolinite took place in the oxalic-acid and H_3PO_4 series. The initial acidity was such that it broke down some of the kaolinite and prevented as much fixation as occurred when only H_3PO_4 of pH 1.9 was used. The oxalic acid had a pH of 1.6 and the pH of the H_3PO_4 and oxalic acid mixtures must have been somewhere between these values.

Decomposition also occurs when the concentration of OH ion becomes sufficient. A 0.1N NaOH solution decomposes kaolinite to a slight extent. When the KOH and 0.1N H_3PO_4 mixture (pH 12.2) was used with

kaolin, a slight decomposition occurred, as evidenced by the fact that a small amount of aluminum went into solution. The equilibrium pH was 10.51. No aluminum was found in the filtrate from the KCl–KOH–H_3PO_4 (pH 10.0) treatment of kaolinite. In this case, however, the final pH dropped to 8.0. The data indicate that a wide ratio of solution to kaolinite and pH values of 10 or above are necessary before there is any appreciable decomposition on the alkaline side. The data indicate that the decomposition of kaolinite occurs at pH values outside the range for agricultural soils or for plant growth. Furthermore, any phosphate the kaolinite fixes, especially from a phosphate solution of low concentration (which would be the case in the soil even with liberal phosphate fertilization), is of low availability for plant use.

The exact effect of the oxalate ion is not clear. Russell and Prescott (*42*) found that dilute acids removed phosphates from a soil in the following order: $0.1N$ oxalic $>$ $0.1N$ citric $>$ $0.1N$ H_2SO_4 $>$ $0.1N$ HNO_3 $=$ $0.1N$ HCl; and that soils adsorbed phosphorus from sodium phosphate readily in the presence of HCl and HNO_3 but to a notably less extent in the presence of an equivalent concentration of citric acid. They infer that acids like citric and oxalic satisfy the adsorption capacity of the soil and leave it with little power to take up phosphoric acid. Demolon and Bastisse (*8*) found that the presence of citrate and oxalate ions reduced the amount of P_2O_5 fixed by a soil. No data were presented to show the fixation of the citrate or oxalate ions though a partition of these ions to the phosphate ion was given as a reason for the lower power to fix phosphate. Lichtenwalner, Plenner, and Gordon (*25*) found that citric acid had no effect in removing phosphate which had been fixed by an iron hydrogel, but it peptized the aluminum hydrogel and removed some adsorbed phosphate from the latter. Weiser (*49*) reported that organic matter was apparently effective in deactivating iron- and aluminum-fixing materials.

The data presented in tables 15 and 16 substantiate the above observations that the presence of oxalate ions (or organic matter) reduce phosphate fixation, but they do not substantiate a strict stoichiometric relation in the fixation process.

COMPARISON OF KAOLINITE WITH OTHER CLAY MATERIALS

One-gram samples of kaolinite and H-bentonite were shaken intermittently for 1 week with 50 cc KH_2PO_4 solution containing 98.55 mg of PO_4. The suspensions were centrifuged, and the clear filtrates were analyzed. One-gram Volclay[n] samples were shaken continuously on a

[n] Volclay is a trade name for bentonite found near Belle Fourche, S. D., and supplied by the American Colloid Company.

roller for 24 hours with a similar solution and allowed to stand 2 days before being centrifuged.

TABLE 18

THE FIXATION OF PHOSPHATES BY DIFFERENT CLAY MATERIALS

Reaction*		PO_4 fixed per 100 grams of kaolinite	PO_4 fixed per 100 grams of H-bentonite	PO_4 fixed per 100 grams of Volclay
Original solution	Kaolinite filtrate			
pH	*pH*	*mg*	*mg*	*mg*
4 54	6.10	3,384	1,510	689
6 08	6 36	3,056	1,407	
6.38	6 54	2,759	1,305	
6 75	6 85	2,452	1,407	516
9 22	7.86	986	762	295

* The pH of the H-bentonite and Volclay filtrates was not determined.

The data on fixation are given in table 18. Apparently kaolinite has a much higher capacity for fixing phosphate than has bentonite. Soils in which the clay is of a kaolinic character would therefore require a higher rate of phosphate fertilization in order to give increased yield if the kaolinite is finely divided.

GREENHOUSE TESTS OF PHOSPHATE FERTILIZER ON AIKEN CLAY LOAM

The Effect of Rate and Placement.—Two different rates of application of superphosphate were used in these experiments—280 pounds and 2,800 pounds per acre. Each 2-gallon glazed earthenware pot contained 8,000 grams of Aiken clay loam. No drainage was allowed to occur from the pots. In one series the phosphate was thoroughly mixed with all of the soil; and in the other series it was mixed with the upper 3,000 grams. The phosphate was applied only once, at the beginning of the experiment. At each planting date, 25 cc of *M* $Ca(NO_3)_2$ and a like amount of *M* KNO_3 were added to each pot. Tomatoes, barley, and vetch were used as the major crops, with buckwheat introduced in cases where the other crops were failures or did not seem to be giving much growth. All treatments were in triplicate.

The plants were harvested when the first blossoms appeared. Only the tops of the plants were used in determining the phosphate removed. The roots were left in the soil.

Tomatoes were a failure with the lower application of phosphate, and barley did not grow very well; hence, buckwheat was substituted later. From the data (table 19), buckwheat appears to be able to do much better than either tomatoes or barley. This is in accord with general field

observations. All of the crops were able to make a fair growth when the rate of application of superphosphate was 2,800 pounds per acre. In a general way, the data bear out the conclusion that the efficiency of superphosphate is slightly higher where locally placed in the soil. The results also show that for some crops a nominal application of superphosphate (280 pounds per acre) on this soil is valueless, and to get response for

TABLE 19

THE RECOVERY OF PHOSPHATE FROM AIKEN CLAY LOAM BY VARIOUS CROPS

(Averages of triplicate treatments)

P applied	Crop sequences*	P recovered		Total P recovered	
		Major crop	Buckwheat	Amount	Percentage†
mg		*mg*	*mg*	*mg*	*per cent*
0	T T Bu Bu	0 07	4 98	5 05	
	B B B Bu	1 61	1.28	2 89	
	V V V V	10.38		10 38	
125.75 mixed throughout soil	T T Bu Bu	0.19	9.51	9 70	3 70
	B B B Bu	15 62	16 00	31 62	22 85
	V V V V	28 46		28 46	14 38
125 75 in upper layer	T T Bu Bu	0 49	11 68	12 17	5 66
	B B B Bu	11 57	9 47	21 04	14 43
	V V V V	31 08		31 08	16 46
1,257.5 mixed throughout soil	Tomatoes (4 crops)	76 54		76 54	6 08
	Barley (4 crops)	67 80		67.80	5 22
	Vetch (4 crops)	105 19		105 19	7 54
1,257.5 in upper layer	Tomatoes (4 crops)	134 65		134 65	10 70
	Barley (4 crops)	109 80		109 80	8 56
	Vetch (4 crops)	119 92		119 92	8 71

* T=Tomatoes, B=Barley, V=Vetch, and Bu=Buckwheat

† The percentage recovery is calculated after deducting the amount of phosphorus present in crops grown on unfertilized soil; in the case of 4 crops of tomatoes or barley, the deduction is based on the assumption that the recovery by 4 crops is proportionate to that by 2 or 3 crops.

these crops, large applications must be made. Investigations (*43*) have shown that for certain soils small applications of soluble phosphate fertilizers give no response, while a much higher rate of application often gives good results.

The Effect of Soil Reaction.—The reaction of the Aiken clay loam was modified by the use of dilute H_2SO_4, $CaCO_3$, and Na_2CO_3. The $CaCO_3$ and Na_2CO_3 were mixed with the soil by rolling the soil back and forth several times. The dilute H_2SO_4 was sprayed on thin layers of the soil; after which the soil was allowed to come to equilibrium with the acid. The acidulation process was repeated until the equilibrium mixtures had the desired pH. Triple superphosphate at the rate of 2,800 pounds per acre was thoroughly mixed with the soil. This was equivalent to

3,142 mg of phosphorus per 2-gallon pot holding 8,000 grams of soil. All treatments were in triplicate.

TABLE 20

THE EFFECT OF REACTION ON THE RECOVERY OF APPLIED PHOSPHATE TO AIKEN CLAY LOAM

(Averages of triplicate treatments)

Reaction of soil	Tomatoes*			Barley*			Vetch*		
	Total yield	P recovered		Total yield	P recovered		Total yield	P recovered	
		Amount	Per cent†		Amount	Per cent†		Amount	Per cent†
pH	*grams*	*mg*	*per cent*	*grams*	*mg*	*per cent*	*grams*	*mg*	*per cent*
4 0	0 00			0.00			0 00		
5 0	44 59	93 71	2 98	46.66	129 21	4 11	17 09	36 21	1 15
5 4	76 95	145 90	4 64	31 77	88 65	2 82	16 81	42 55	1.35
6 0‡	74 75	166 12	5.29	72 75	165 61	5 27	32 47	65 90	2 10
7 8									
$CaCO_3$§	70.53	158 17	5 03	66 93	163 80	5 21	26 54	57 34	1.82
Na_2CO_3¶	36 14	81 12	2 58	73 07	131 94	4 20	12 11	30 89	0.98

* In all cases, except for tomatoes in the pH 5.4 series, 3 crops were grown In the exception noted, only 2 crops were grown during the period of the experiment.

† On the basis of 3,142 mg of phosphorus added per pot. No deductions were made for the soil, since other experiments indicated that this amount was negligible.

‡ Natural soil § 80 grams per pot. ¶ 400 cc *M* Na_2CO_3 per pot.

Where Na_2CO_3 was used, the soil had an undesirable physical condition which tended to prevent water penetration. As a result, aeration was deficient, especially after the addition of moisture to the soil. The data in general (table 20) indicate that within the reaction range suitable for crop production, there is little to choose. Apparently a reaction between pH 6 and pH 7 is most satisfactory, and nothing would be gained in the Aiken clay loam by changing the present soil reaction.

TABLE 21

THE EFFECT OF SOURCE OF NITROGEN ON THE RECOVERY OF PHOSPHORUS FROM TRIPLE SUPERPHOSPHATE BY TOMATOES

(Averages of triplicate treatments)

Treatment	Yield			Total P recovered	
	First crop	Second crop	Third crop	Amount	Per cent*
	grams	*grams*	*grams*	*mg*	*per cent*
Triple superphosphate only	23 72	3 44	4 82	107 02	3 41
Triple superphosphate + $(NH_4)_2SO_4$	34.66	26 02	7 72	156 13	4 97
Triple superphosphate + $Ca(NO_3)_2$	36 91	29 92	11 76	159 91	5 09

* On the basis of 3,142 mg of phosphorus added per pot No deductions were made for that furnished by the soil without fertilization since other experiments indicated this was negligible.

The Effect of Source of Nitrogen.—Triple superphosphate at the rate of 2,800 pounds per acre was thoroughly mixed with virgin Aiken clay

loam. This was equivalent to 3,142 mg of phosphorus per 2-gallon pot holding 8,000 grams of soil. In some cases, the nitrogen was supplied as

TABLE 22

THE EFFECT OF A GOOD STARTING MEDIUM ON PHOSPHATE REMOVAL BY TOMATOES
(Averages of triplicate treatments)

Series	Medium of growth*	Yield			P in crops
		First crop	Second crop	Third crop	
		grams	*grams*	*grams*	*mg*
1	5,000 grams Aiken clay loam beneath 3,000 grams Fresno .	22 91	16 42	9 64	89 64
2	5,000 grams sand† beneath 3,000 grams Fresno fine sandy loam	14 50	10 37	3 22	56 08
3	8,000 grams Aiken clay loam	0 02	0 05	0 05	0 10
4	8,000 grams Fresno fine sandy loam .	25 29	26 96	14 82	178 92

* Each pot received 25 cc of *M* $Ca(NO_3)_2$ applied to the surface at each planting date.

† At the beginning of the experiments and at the beginning of each succeeding cropping period, 500 cc of a solution 0.005 *M* in $Ca(NO_3)_2$, 0.005 *M* in KNO_3, and 0.002 *M* in $MgSO_4$ were added to the sand; 2 cc of iron tartrate were also added.

$(NH_4)_2SO_4$, and in others as $Ca(NO_3)_2$. At the beginning of growth at each planting date, 25 cc of *M* $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ was added. All treatments were in triplicate. Tomatoes were grown on the soil.

Where the triple superphosphate was used alone, the foliage had a yellow color, which indicates nitrogen deficiency, especially after the first period. Even the first crop showed some nitrogen deficiency. The

TABLE 23

THE RECOVERY BY TOMATOES OF PHOSPHORUS SUPPLIED TO AIKEN CLAY LOAM BY SUPERPHOSPHATE OF DIFFERENT PARTICLE SIZES
(Averages of duplicate treatments)

Size of particle	Oven-dry weight of crops	Applied phosphorus recovered by plants	Ratio to granulated super-phosphate
	grams	*per cent*	*1 00*
Granulated superphosphate	26 13	7.32	1 00
Up to 10 mesh	40 45	16 22	2 21
10 to 20 mesh	33 07	11 95	1 63
20 to 60 mesh	21 80	5 90	0.80
60 to 100 mesh	18.75	5 09	0 69
100 to 200 mesh	10 89	2 31	0 31
Finer	11.65	3 23	0 44

influence of the triple superphosphate extended over two cropping periods where the nitrogen deficiency was corrected (table 21), but beyond this the applied phosphate had little effect. The third crop in all cases was very spindly and showed phosphate deficiency. No choice can be made between the use of $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ as the source of nitrogen with triple superphosphate on this soil.

The Effect of a Good Starting Medium.—Three crops of tomatoes were grown in pots containing a total of 8,000 grams of Fresno fine sandy loam, Aiken clay loam, or combinations of these with each other or with sand. Series 1 consisted of 5,000 grams of Aiken clay loam placed in the bottom of the pot; on top of and unmixed with this were 3,000 grams of

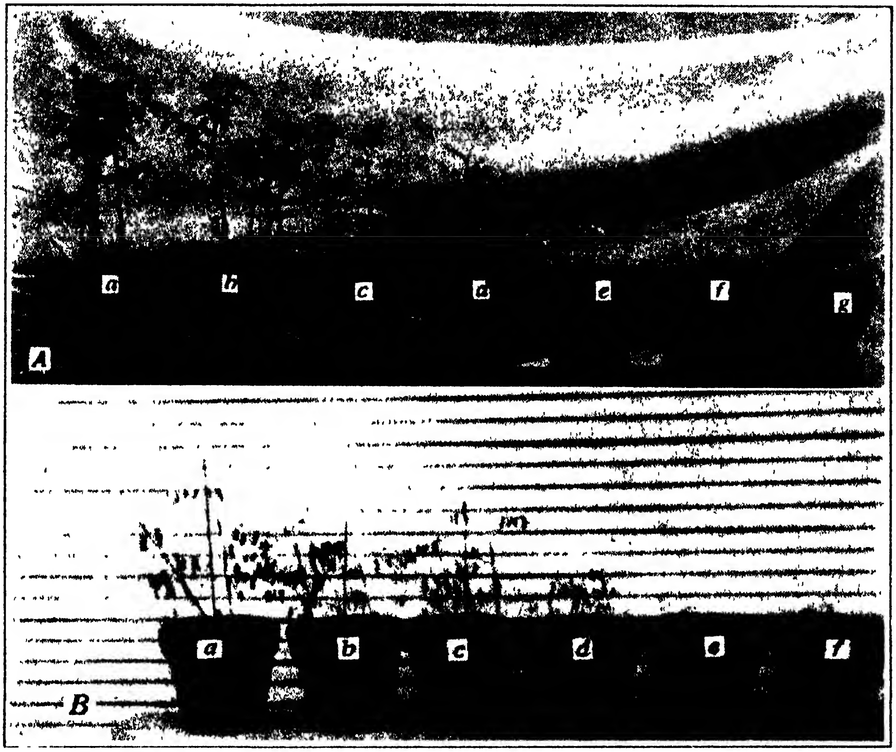

Fig. 6.—The influence of the size of superphosphate particles on the growth of tomatoes on Aiken clay loam: *A*, second crop; *B*, sixth crop. The size of particles of superphosphate applied to the various pots was as follows: *a*, coarser than 10 mesh; *b*, 10–20 mesh; *c*, 20–60 mesh; *d*, 60–100 mesh; *e*, 100–200 mesh; *f*, finer than 200 mesh. In series *A*, pot *g* contained Aiken soil with no phosphate.

Fresno soil. Series 2 consisted of 5,000 grams of acid-washed white sand in the bottom of the pot; on top of and unmixed with this were 3,000 grams of Fresno soil. Series 3 was Aiken soil only. Series 4 was Fresno soil only. The Fresno and Aiken soils contained 0.16 per cent and 0.18 per cent of total PO_4 respectively. All treatments were in triplicate.

The tomato plant was unable to make any growth on the Aiken soil, but it made a nice growth on the Fresno soil (table 22). The root systems were extensive both in the Aiken soil below the Fresno soil and in the sand below the Fresno soil; more phosphorus was recovered from the plants on the former than on the latter, but just how much of the extra

phosphorus was taken from the Aiken soil cannot be determined from these data. The results indicate that if the plant is given a good start, it can probably secure more phosphorus than it normally does from unfertilized virgin Aiken soil.

The Effect of Size of Superphosphate Particles.—Aiken clay loam in 7,000-gram lots was placed in 10-inch pots and 2.8 grams of superphosphate of various-sized particles were thoroughly mixed in the upper 2,000 grams of soil. Nitrogen was supplied in the form of $Ca(NO_3)_2$ and KNO_3 before planting each crop. Seven consecutive crops of tomatoes were grown in each pot without any further phosphate treatment. Treatments were in duplicate.

Table 23 gives the condensed results. They indicate clearly that coarse particles are to be preferred over fine particles of superphosphate for such soils (fig. 6).

GREENHOUSE TESTS OF COMPARATIVE VALUE OF SUPERPHOSPHATE AND "KAOLINITE PHOSPHATES"

Samples of ground kaolinite were treated with different strength solutions of KH_2PO_4. After several hours of shaking, the kaolinite complex was thoroughly washed with distilled water to remove any occluded phosphate, and the PO_4 content of all of the leachates was determined. The difference between the PO_4 in the original solution and that found in the leachate was considered as fixed phosphate. The term "kaolinite phosphate" is used to designate the phosphated kaolinite. Three different degrees of saturation were secured with kaolinite treated as given above. These were used in sand cultures and compared with the phosphorus furnished by 1 gram of superphosphate. The amount of phosphorus furnished in all cases was made equivalent to that in the superphosphate by adjusting the amount of phosphated kaolinite. Each pot held 5,000 grams of pure sand. The phosphate was thoroughly mixed throughout the sand. To each container, after planting tomato seed, was added 800 cc of Hoagland's solution minus PO_4. Five plants were allowed to grow in each pot. The growth period was of 45 days' duration (January 7 to February 21, 1938). The plants grown on the superphosphate treatment were in bloom at the time of harvest. The oven-dry weights of the plants were as follows:

Phosphate used	Total weight of plants, grams
Superphosphate	8.10
"Kaolinite phosphate," 64.0 mg PO_4 per gram	2.40
"Kaolinite phosphate," 36.0 mg PO_4 per gram	0.95
"Kaolinite phosphate," 14.5 mg PO_4 per gram	0.45
None	0.14

The comparative growth with the various treatments is shown in figure 7.

The data indicate that the availability of the adsorbed phosphate varies with the degree of saturation of the adsorbed complex. If a light

Fig. 7.—The comparative growth of tomatoes in sand cultures where the phosphorus is furnished as: *a*, superphosphate; *b*, strongly saturated "kaolinite phosphate"; *c*, medium-saturated "kaolinite phosphate"; *d*, weakly saturated "kaolinite phosphate."

fertilizer application is made on a soil, the phosphate of which is controlled by the kaolinite complex, there will be practically no response by certain crops. Only when the kaolinite is more highly saturated will the plant be able to secure its needed phosphate for growth, and even then the growth period must be of considerable length in order to secure full development of the plant.

GREENHOUSE TESTS OF THE EFFECT OF VARIOUS COLLOIDS ON SUPERPHOSPHATE EFFICIENCY

Greenhouse experiments were conducted with sand mixtures and Aiken clay loam mixtures in Mason quart jars. The total air-dry weight of material in each jar was 1,250 grams except in the case of the Aiken soil, where it was 860 grams. The treatments were conducted in duplicate. The colloid and superphosphate were thoroughly mixed in the jar. The kaolinite used in the 1 per cent mixture had been ground in a ball mill for 7 days. The Volclay was 200-mesh material. The Aiken colloid had been pulverized in a mortar until it was quite fine. Natural Aiken clay loam was used without any previous treatment. One tomato plant was grown per jar. At the beginning of the experiment, 200 cc of Hoagland's solution minus PO_4 was added to each jar.

The results (table 24 and fig. 8) fully justify the conclusion that kaolinic clays are very effective in fixing phosphates in a form not readily

accessible to plants when limited amounts of soluble phosphates are applied. On the other hand, a soil containing montmorillonitic (bentonitic) clay should be more effective in supplying plants with their needed phosphate. The plants in the sand culture were in bloom when harvested.

TABLE 24

EFFECT OF VARIOUS COLLOIDS ON GROWTH OF TOMATOES WITH VARIOUS ADDITIONS OF SUPERPHOSPHATE

Culture	Superphosphate additions*				
	None	50 mg	100 mg	150 mg	200 mg
	Average total oven-dry weight of tomato plants				
	grams	*grams*	*grams*	*grams*	*grams*
Sand only	0 02	1 00	1 60	1 80	1.75
Sand + 1 per cent Volclay	0 02	1.45	1.45	1 40	1 50
Sand + 1 per cent Aiken colloid	0 01+	0.13	0 90	1 42	1 65
Sand + 20 per cent crushed kaolinite	0 02	0.02	0.02	0 02+	0 04
Sand + 1 per cent finely divided kaolinite	0 01	0.01+	0.02	0 03	0 03
Aiken clay loam.	0 01	0 01+	0 02	0 03	0 03

* Considering the volume weights of sand and Aiken clay loam, these applications of superphosphate are approximately equivalent to 80, 160, 240, and 320 pounds per acre, respectively

DISCUSSION OF RESULTS

Certain soils contain colloidal material which by X-ray pattern analysis has been shown to be kaolinic in character. Other investigations, where X-ray facilities have not been available, have shown the colloidal fraction of a great many soils to have a low silica : sesquioxide ratio, suggesting the presence of a kaolinic type of clay. These soils are known to have a high capacity to fix soluble phosphate fertilizers in forms not readily available for plants. Since most of these soils are red in color, they have been assumed to be high in iron. In some cases this is actually the case, but in others the iron content is not so great as the color of the soil would indicate. Iron has been considered the chief factor in the phosphate-adsorbing system of these soils. Certain results render such an assumption questionable.

The presence of free ionic iron in any appreciable amount in the soil occurs only under extreme conditions of soil reaction. Much the same may be said of aluminum. Experiments show (curve *b* in figs. 1 and 2, pp. 348 and 349) that iron phosphate formed by these ions would be removed from solutions within the usual reaction range of soils. But such experiments do not prove that ionic iron is present in soils to fix applied soluble phosphates rapidly; and hence, do not give the real picture of existing soil conditions.

Iron and aluminum hydrosols and hydrogels are known to have the ability to remove soluble phosphates from solution under certain conditions (curve *a* in figs. 1 and 2). Hydrolysis of the freshly prepared iron complexes occurs, however, and at pH 6 and above there is a considerable release of PO_4 even in the presence of an excess of iron. The PO_4 released under these conditions by the hydrosol is much greater than that present

Fig. 8.—The comparative growth of tomatoes as influenced by phosphate availability in: *a*, sand only + 200 mg superphosphate; *b*, sand + 1 per cent Volclay + 200 mg superphosphate; *c*, sand + 1 per cent kaolinite + 200 mg superphosphate. (Other nutrients were derived from Hoagland's solution minus PO_4.)

in the soil solution under similar conditions, and is not in accord with the ability of plants to grow on such soils. Other factors must, therefore, be involved.

The experiments with a good grade of kaolinite as shown by X-ray pattern data (fig. 3, p. 357) indicate that this material has a high phosphate-fixing power when ground in a ball mill for a few days. The clay particles of soils are colloidal in nature and it is permissible to assume that at least some of the kaolinite is colloidally dispersed. Russell (*41*) states that under natural conditions kaolinite is not so finely divided as some of the other clay minerals. The data in table 8 (p. 357) indicate that a soil weighing 2,000,000 pounds per surface 6 inches in depth and containing 1 per cent of finely divided kaolinite would be able to fix 2,058 pounds of PO_4 under conditions similar to those in the experiment.

The fixation by kaolinite is extremely high for all concentrations of soluble phosphates in the reaction range of agricultural soils. However, the product formed varies in phosphate saturation according to the concentration (amount of phosphate per unit volume of solution) of phosphate at its disposal. When these "kaolinite phosphates" of different degrees of saturation are used as a source of phosphate for plants in pot cultures, the response is directly proportional to the degree of satura-

tion. This means that where this kind of adsorbing system is controlling the phosphate equilibrium in the soil, a small application is ineffective; and to secure results, a much higher rate of phosphate application must be employed.

The fixation is greatest at acid reactions. When the exposed solution contains H_2PO_4 ions, the fixation is great. This has a very important bearing, because experiments (*23, 32*) indicate that this phosphate ion is the one which plants absorb most rapidly. If the colloidal system of the soil prefers this kind of ion for fixation, soils having this kind of adsorbing system are extremely efficient in "competing" with the plant for phosphorus. As a result, the plant, because of its limited root area, is at a great disadvantage and suffers because of this competition.

Decomposition data indicate that the valences necessary for the fixation depend upon aluminum. When the reaction is such that much aluminum appears in solution, the fixation of phosphate by the kaolinite is decreased. This, however, does not take place in the usual soil reaction range and although of theoretical value in determining the nature of the fixation is not of practical agricultural interest. When the concentration of the OH ion is increased, less and less phosphate is fixed. When the OH ion concentration attains a certain strength, somewhere above pH 10, there is little or no more fixation; but an actual breakdown of the clay complex occurs. Likewise, if the H ion concentration is increased sufficiently, somewhere below pH 2, decomposition occurs, as would be expected from the use of strong acids in general analytical work. Between these extremes, the phosphate fixed increases with a decrease in pH, and cation fixation decreases.

Colloids having a high silica:sesquioxide ratio have a lower capacity to fix phosphates, as shown by both chemical and greenhouse work. This is in general accord with the work of other investigators.

Gile (*18*) found in studying the influence of various soil colloids on the efficiency of superphosphate that soils having colloids of low silica: sesquioxide ratios depressed the efficiency more than those having a higher ratio. Furthermore, the colloids which depressed superphosphate most were those of smallest base-exchange capacity. These properties suggest that the high phosphate-fixing colloids studied by Gile were kaolinic in character.

Kaolin is formed by weathering processes (*39*) and is probably present in some of its forms in all soils containing colloids of a low silica:sesquioxide ratio. Not only have such soils, therefore, active sesquioxides in more or less abundance, but also the kaolin in them is in a highly divided state as a result of the pronounced weathering, which augments phos-

phate retention greatly. The presence of kaolin in this condition severely handicaps the ability of plants to secure phosphorus either from the supply in the soil itself or from that of applied soluble phosphates, which are rapidly converted into a highly inaccessible form.

The other clay minerals studied exhibit less fixing power, but even so their presence in soils is an important contributing factor to phosphate fixation. According to our investigations, the efficiency of applied soluble phosphates is a function of the kind of clay minerals present in the soil: if the clay is kaolinlike, a low efficiency is inevitable, while soils with a montmorillonitic clay complex will have a higher degree of phosphate efficiency.

Coarsely ground superphosphate is better than finely divided superphosphate for these kaolinic soils. There are two reasons for this: First, such material is less rapidly fixed in the soil; second, the fixed phosphate is in more highly saturated zones, and as such, is of higher availability than it would be if it were fixed from finer phosphate particles, where the concentration of the solution surrounding the clay is lower. The first reason accounts for the better results in the first crops, and in all probability the later crops are benefited largely because of the second factor. Local application also would have produced more highly saturated colloids.

SUMMARY

Soils with a kaolinic type of clay have a high capacity to fix soluble phosphates, as is shown by both chemical and greenhouse experiments. This is in accord with field results.

Phosphate fixation by the various iron systems alone is not considered sufficient to account for all of the properties that these soils show with respect to phosphate availability.

Kaolinite when ground to colloidal dimensions has a high phosphate-fixing capacity.

The fixation is greatest at acid reactions, which indicates that the H_2PO_4 ion is the most favored phosphate ion for the reaction.

An increase in base-exchange capacity accompanies the increase in phosphate fixed.

Greenhouse experiments show that the availability of the phosphate in "kaolinite phosphate" is directly proportional to the degree of phosphate saturation. This is taken to indicate that a light phosphate fertilizer application would be ineffective on soils having a kaolinic kind of clay.

Even a very low percentage of kaolinite, if in a colloidal condition in a soil, will tie up phosphate and make it unavailable for plants.

Greenhouse results substantiate the laboratory data in showing that bentonite (Volclay) has a much lower capacity to fix phosphorus in an inaccessible form for plant growth than does colloidal kaolinite.

The results are considered sufficient to explain why soils with a kaolinic type of clay have a high capacity to fix phosphate and a low phosphate availability as measured by plant growth.

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WATER CONDUCTION FROM SHALLOW WATER TABLES[1, 2]

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INTRODUCTION

THE PHENOMENON of the flow of liquids through porous mediums without the application of external force and without complete filling of the pores of the solid with liquid, has long been recognized and studied. It was early recognized that the forces involved are those of adhesion and cohesion, the same as those responsible for the action of liquids in capillary tubes. The term "capillarity" (*6, 17*)[4] has thus come to apply to the flow of liquids through porous mediums.

Many analyses (*28–31*) have been made for the purpose of evaluating the capillary forces acting in three-phase systems, such as is the case in moist soil, consisting of solid, liquid, and gas, by assuming spherical solid particles of uniform size and sequence of packing arrangement. While this assumption has presented concepts of value in comprehending the mechanism involved in capillary flow, the size and configuration of the solid and liquid phases are very complex in even the most idealized systems, and become indeterminate when applied to natural bodies such as soil.

The capillary potential concept, introduced by Buckingham (*6*) in 1907, assumed a capillary force field generated by the attraction of moist soil for water. He defined a capillary potential, the gradient of which is equal in magnitude to the capillary force. The introduction of the potential function gave rise to the study of soil-moisture as a dynamic system; but this method received no added impetus until 1922, when Gardner (*9*) and others showed that the capillary potential of Buckingham may be

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[2] Abridged from a thesis submitted in partial fulfillment of the requirements for the degree of doctor of philosophy.

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[4] Italic figures in parentheses refer to "Literature Cited" at end of this paper.

considered as a pressure potential due to the differential pressures on either side of the liquid-gas interface in the menisci of the water films. They further showed it to be directly measurable, over a certain range of potential, by measuring the negative hydrostatic pressures within the water films of soil moisture. The instrument used for these direct measurements was called a capillary potentiometer, but is now called a tensiometer (*19*), and consists of a porous absorbing element, an adaptation of the Livingston Auto Irrigator, to which a manometer is attached. When the capillary potentiometer was filled with water and the porous absorbing element was embedded in the moist soil whose capillary potential was to be measured, water transfer took place between the porous element and the soil until, at equilibrium, the pressure of the water inside the potentiometer was equal to the pressure in the soil-moisture films. This pressure was read directly on the manometer.

In the application of the dynamic concept to soil moisture studies, the velocity of flow of water through the soil is considered to be proportional to the total water-moving force. A conductivity factor, variously called capillary conductivity, capillary transmission constant, conductivity, and permeability, has been used to express this proportionality (*3, 7, 8, 10, 16*). The term "permeability" is adopted in this paper.

Many data on the permeability of soils in saturated flow or, with the pore spaces entirely filled with water, are available in papers of the U. S. Geological Survey, the American Geophysical Union, and in engineering papers. Slichter (*24*) made theoretical calculations for the flow of underground water under pressure, in which it was assumed that the velocity of flow was proportional to the pressure gradient. There are relatively few published data on soil permeability in unsaturated flow, however. Such data as have been reported were derived from experimental results on relatively small quantities of soil through which flow was induced by artificially maintaining differential pressures in the moisture films on either side of the sample. Richards (*17, 18, 20*) has published data on three soils, including capillary potential as a function of water content, permeability as a function of water content and capillary potential, and permeability of a peat soil as a function of capillary potential.

The evaluation of the movement of water through unsaturated soil is important in many practical problems, such as: the drainage of land, of road subgrades, and of all structural foundations and pavements laid on the ground; the contribution of a water table to the water supply of plants; the loss of water from a soil surface by evaporation; and the upward translocation and concentration of soluble salts in the soil.

This paper reports pressure potential and saturated and unsaturated permeability data, using six California soils. The rate of water flow required to maintain shallow water tables in cylindrical soil columns 8 inches in diameter, was measured in graduated supply burettes. Unsaturated flow was induced naturally; the water rose from the water table to the surface of the soil columns by capillarity, and was evaporated from the surface. Tensiometers were spaced at regular intervals on the vertical axis of the soil columns, and the pressure potential values were read directly on these instruments. When the rate of water uptake and the pressure potentials throughout a soil column became steady, that column was said to be at steady state. Its moisture distribution was then determined by sampling, and its saturated and unsaturated permeabilities at various pressure potentials were determined from the velocity of flow and the total potential gradient.

PROCEDURE

The moisture studies reported herein were carried out in a room in a light frame building of the University of California at Berkeley. The room was 8 × 16 feet, and 12 feet high, with a reinforced concrete floor laid on the ground. The walls were of tongue-and-groove pine sheathing on 2 × 4 inch studs spaced at 2-foot centers, the naked studs being on the room side. After beginning this experiment, the walls and ceiling were lined with celotex wallboard which was nailed to the studs.

The room was heated by two batteries of electrical heating elements, one battery placed at either end of the room about 4 feet from the end walls and 6 feet from the floor. Heat distribution was effected by four electric fans, so placed as to give maximum air turbulence as well as general air circulation to all parts of the room.

The heating elements had two circuits, one continuous and one intermittent. The intermittent circuit was opened and closed by a thermal regulator through a relay. Temperature at the thermal regulator was set at 30° C. and controlled to about ± 0.02°. Temperature along the sides of the room and near the floor could not be held to this narrow range due to heat loss through the rather poorly insulated walls. The soil columns, which were set in a row close to a wall, normally held to a temperature range of 0.02° C. except during periods of abnormal change in the atmospheric temperature outside the room.

Six soils were used, ranging in texture from sand to clay (table 1). All except one, the Oakley sand, were of the Yolo series. The air-dry soils were prepared for filling the cans by breaking them down to pass a 3-mm screen.

The soil cans were of galvanized iron, cylindrical, 8 inches in diameter and 3 to 4 feet high (fig. 1), and were fitted with a wire-screen diaphragm soldered 2 inches above their bases to provide support for the soil columns. A water inlet tube, ¼ inch in diameter, was soldered 1 inch above the bottom of each can. Each can was punched with ⅝-inch holes in four vertical rows, one row at each quarter point on the can's circumference. The vertical distance between holes in the row and the elevation of each row, were arranged to give a hole for each inch of can height. Before a can was filled with soil the holes were closed with patches of celluloid cemented to the outside. When the wetting front in the soil column reached a hole, the celluloid patch was removed, the soil sampled for moisture content, and the hole reclosed with a rubber stopper.

TABLE 1

Mechanical Analysis and Moisture Equivalents of the Soils Used

Separates	Oakley sand	Yolo sand	Yolo fine sandy loam	Yolo clay loam	Yolo light clay	Yolo clay
Fine gravel	0.2	2.9	0.1	0.1	.	.
Coarse sand	9.9	16.4	0.3	0.5	0.1	0.2
Medium sand	15.3	25.8	0.5	0.6	0.4	0.3
Fine sand	45.1	41.8	18.9	5.3	7.2	3.0
Very fine sand	20.4	6.0	31.0	21.0	16.1	8.1
Total sand	90.9	92.9	50.8	27.5	23.8	11.6
Total silt (by difference)	3.5	3.3	31.5	46.0	45.0	46.5
Total clay	5.6	3.8	17.7	26.5	31.2	41.9
Clay <2 micron	4.6	3.2	12.8	17.2	23.2	33.1
Clay <1 micron	4.0	2.9	9.4	13.5	17.4	26.5
Moisture equivalent	4.3	3.5	18.1	22.5	25.0	26.3

The procedure in filling the can with soil was similar to the tremie method for placing concrete under water (*36*). A strong fiber tube, 4 inches in outside diameter, and 4 feet long, originally made for the packing and shipment of glass tubing, was used as a tremie.

The tremie tube, surmounted by a funnel, was placed upright in the soil can. One man kept the funnel and tremie full of soil and a second man operated the tremie, partly supported its weight, kept it vertical, and moved it with a rotary motion so that the tube described a circle, the diameter of which was equal to the diameter of the soil can. The bottom of the tube, resting lightly on the soil, passed over the entire area of the soil column at each revolution, and thus maintained a level and regular surface. Approximately eight revolutions of the tremie were required per inch of depth of soil column laid down.

The entire system, can, tremie, and soil, was weighed at intervals of approximately 4 inches in the soil-column height. For the height of soil

at each 4-inch stage the mean of eight to ten measurements was taken; these measurements were made from the top of the can with a meter stick which was dropped vertically onto the soil through a distance of ap-

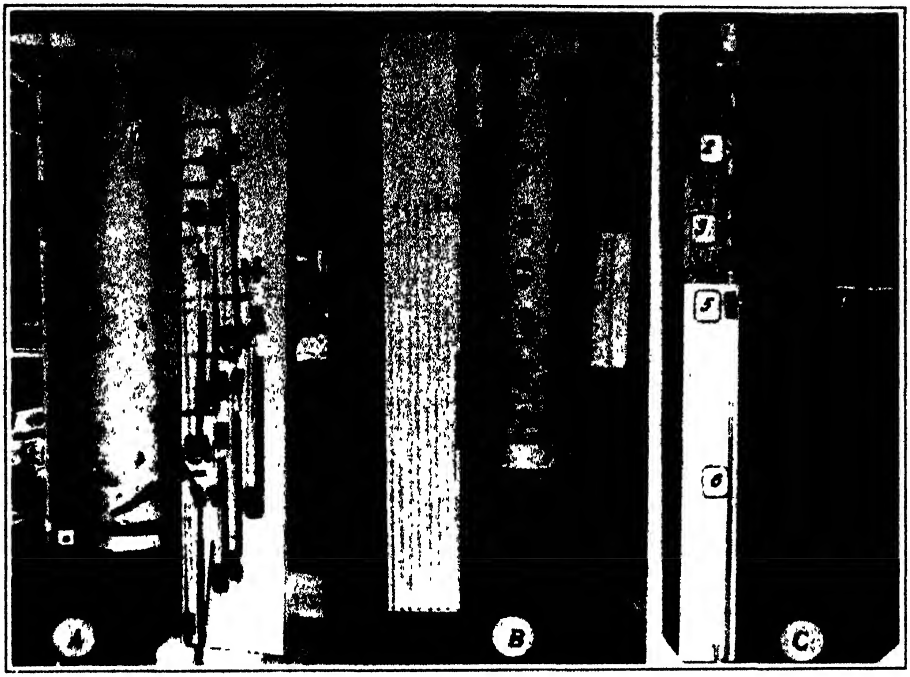


Fig. 1.—Soil cans with tensiometers installed.

A, Can with mercury tensiometers, showing the method of attaching the manometer panel and the installation of the tensiometers. The instrument at the top of the panel is a vibrator. The lowest tensiometer has been removed from the soil to show the cup (absorbing element).

B, Can with water tensiometers installed. Eight tensiometers with water manometers are mounted on the left panel. The tensiometers enter the soil column back of the panel. Two open water manometers used for positive potentials only are mounted on the small panel at the right.

C, Assembly of the tensiometer with a mercury manometer. This assembly was used for testing tensiometer cups and is the same as the tensiometers installed in the soil columns except that the stopcock, *3*, was replaced by a screw clamp. Details are as follows: *1*, A three-way stopcock sealed to a male standard taper ground glass connection. The two rubber tubing lines supply vacuum and water at atmospheric pressure. *2*, Female standard taper ground glass connection. *3*, Two-way stopcock. *4*, Porous fired-clay tensiometer cup. *5*, Screw clamp. *6*, Capillary staff of mercury manometer.

proximately 1 inch. The net weight of soil in the can was later corrected for air-dry water content.

Too few weighings were made on an individual soil column to allow a statistical calculation of variation in apparent density for a single column. A mean apparent density was calculated for each column, and

the densities for each stage of filling were expressed as percentages of that mean. These percentages were collected for all the soil columns, analyzed statistically, and a single standard deviation was calculated and expressed as per cent variation from the mean apparent density. This single value was assumed to represent the standard deviation in apparent density for all the soil columns (table 2).

Water at constant pressure was supplied to the base of the columns by water supply units (fig. 2), carried upward through the soil by capillarity, and removed from the soil surface by evaporation. The rate at which water was taken up by the soil was measured in the graduated supply burettes of the water supply units, and the upward advance of the wetted front was observed through the celluloid-covered holes in the sides of the cans.

TABLE 2

Characteristics of the Soil Columns

Soil type and can number		Length of soil column, centimeters	Cross section, square centimeters	Wetting time, days	Drying time, days	Mean apparent density	Standard deviation of apparent density	Standard deviation of mean apparent density	Per cent pore space	Per cent water at saturation by weight, oven-dry basis
Oakley sand, 3		117	314	312	9	1.48	0.042	0.004	43.3	29.2
Yolo sand, 13		84	322	108	5	1.49	.042	.005	43.0	28.8
Yolo fine sandy loam	1	117	314	286	72	1.28	.036	.004	51.0	39.8
	17	84	322	21	7	1.24	.035	.004	52.5	42.3
	20	84	323	64		1.25	.035	.004	52.1	41.6
Yolo clay loam	14	84	321	98	14	1.34	.038	.004	48.6	36.2
	19	84	322	25	3	1.28	.036	.004	51.0	39.8
Yolo light clay	2	117	314	310	34	1.32	.037	.004	49.5	37.5
	18	84	320	25		1.29	.036	.004	50.5	39.1
	20	84	323	29	3	1.27	.036	.004	51.3	40.4
Yolo clay	15	84	322	89	13	1.28	.036	.004	51.0	39.8
	16	84	312	37		1.22	0.034	0.004	53.2	43.5

Tensiometers (*19*), consisting of porous fired clay absorbing elements connected to vacuum gauges of the manometer type, were placed in the soil columns one above the other at intervals of 10 centimeters (fig. 1). A transfer of water takes place between the soil and the absorbing element until, at equilibrium, the pressure of the water in the absorbing element is equal to that in the moisture films of the soil. This pressure is calculated from the height of mercury or other manometer liquid used in the vacuum gauge.

Fig. 2.—Battery of eight water supply units. The details are: *1*, Graduated burettes; *2*, constant-level reservoirs; *3*, air vents; *4*, water supply line from 20-liter supply bottle; *5*, suction line from 20-liter supply bottle; *6*, water lines from constant-level reservoirs to the soil columns; *7*, screw clamp cut-offs on water lines to the soil columns; *8*, screw clamp cut-offs on suction line; *9*, screw clamp cut-offs on water line from the 20-liter supply bottle; *10*, two-way stopcock on water line from 20-liter supply bottle; *11*, two-way stopcock on suction line from 20-liter supply bottle; *12*, water trap in the suction line; *13*, graduated staff tube for measurement of the outflow required to make burette, 1, "gurgle"; *14*, water outlet, used to drain water out of the system.

A burette was filled by closing *7* and opening *8* and *9*. By manipulating *10* and *11*, water flowed through *4* into the constant level reservoir, *2*, and was drawn up into the burette, *1*, by suction on the line, *5*. In operation, with water being supplied to the soil columns, *8* and *9* were closed and *7* was open. Two tubes ran through a 2-hole stopper from *1* into *2*. The lower ends of these tubes were at different elevations so that one tube was continuously immersed in water in *2*, and the end of the other controlled the variation in the elevation of the water level in *2*. When the water level dropped in *2*, air was admitted into *1* through the higher tube and water flowed from *1* to *2* through the other tube, raising the water level in *2* until the end of the higher tube was again immersed and the air supply to the burette was cut off. This cycle was called a "gurgle." At each "gurgle" about 6 cc of water flowed from *1* to *2*, causing a momentary rise of the water surface in *2* of about 0.5 cm. This was sufficient to cause a fluctuation of about 0.4 mm in the water table in the soil column, therefore a water table maintained by this method was relatively constant.

Both negative and positive pressure potentials of the water in the soil columns were measured with tensiometers. Open water manometers of the staff-gauge type were also used in some of the columns for the measurement of positive potentials. For the purpose of these measurements, the pressure potential of the atmosphere was arbitrarily taken as zero.

When the flow of water through the soil columns attained steady state, as indicated by a constant rate of water uptake and steady pressure potentials at each point of measurement, the soil was sampled for moisture content. The steady-state potentials and moisture contents were tabulated and represented graphically. The graphed data (figs. 9–13) include two wetting curves for each soil column:

(1) Moisture content as a function of elevation above the base of the soil,

$$P_w = f(H).$$

(2) Pressure potential as a function of elevation above the base of the soil,

$$\psi = F(H),$$

where: P_w is the per cent water in the soil on the oven-dry basis determined by drying for 24 hours at 105° C. H is the elevation in centimeters above the base of the soil column. ψ is the pressure potential in gram-centimeters per gram (in this paper abbreviated as gm–cm/gm).

After pressure potential and moisture content data had been collected for the soils wetting up, the water supply was removed and a soil-air interface was maintained at the base of the soil columns. During drying, the moisture density throughout the soil columns was reduced by continued evaporation from the surface and by downward flow of water. When the rate of moisture density change and pressure potential change had reached low values at all points in the soil columns, the soils were again sampled for moisture content and pressure potential readings were taken. These data were also tabulated and graphed as $P_w = f(H)$ and $\psi = F(H)$, drying.

The collection, tabulation, and graphical representation of all primary experimental data were included in the procedure outlined above. Later analysis of the primary data includes, first, an examination of the manner in which the pressure potential of a soil is affected by its moisture content, $\psi = f(P_w)$, and, second, an examination of the manner in which the soil permeability is affected by its pressure potential $K = F(\psi)$.

PRIMARY EXPERIMENTAL DATA

In this section are presented the quantitative experimental data discussed in the following order:

1. Mechanical analysis and moisture equivalent of the soils (table 1), and such characteristics of the soil columns as type, apparent density, and per cent pore space (table 2).

2. The manner in which pressure potentials and rate of water uptake vary with time during the wetting-up period (figs. 3 and 4).

3. The manner in which pressure potentials vary with time during the drying period (figs. 5 and 6).

4. The effect of temperature change on pressure potentials and the rate of water uptake in a soil column at steady state (fig. 8).

5. Pressure potentials and moisture distribution in soil columns at steady state (table 3 and figs. 9–13).

Soils Used.—The six soils used in the experiment were Oakley sand, Yolo sand, Yolo fine sandy loam, Yolo clay loam, Yolo light clay, and Yolo clay. All except the Yolo sand were collected from cultivated fields at depths of 2 to 8 inches. The Yolo sand is a stream-washed sand of the same origin as the Yolo soils and was collected in a commercial sand pit on the bank of Cache Creek.

The mechanical analysis of the soils was made by the pipette method using H_2O_2 and HCl pretreatment, and $Na_2C_2O_4$ as a dispersing agent.

The moisture equivalent determinations were made in duplicate on 30-gram samples of air-dry soil crushed to pass a 2-mm sieve. The samples weighed into the cups were moistened for 24 hours, drained for half an hour, and centrifuged for half an hour at a speed sufficient to develop a centrifugal force of 1,000 times gravity.

Mechanical analysis and moisture equivalent data are given in table 1. Numerical data descriptive of the soil in the columns are listed in table 2.

Pressure Potentials and Rate of Water Uptake as Functions of Time During the Wetting-Up Period.—The rate of water uptake as it decreased with time during approach to steady state was taken for all the soil columns. In figure 3 the log (rate of water uptake) is plotted as a function of the log (elapsed uptake time), log $A = f(\log t)$, for Yolo clay, wetting. This curve is similar in form to those of all the soils studied in this experiment and conforms to observed data reported elsewhere (*35*) in the literature of which the example cited is only one of many.

An empirical equation for water uptake derived for a curve expressing

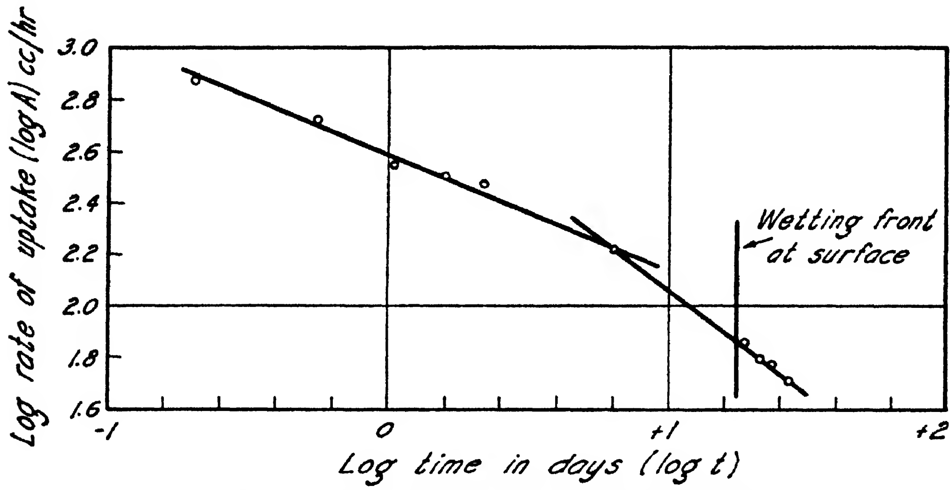


Fig. 3.—Rate of water uptake during approach to steady state, log $A = f$ (log t), for a column of Yolo clay wetting.

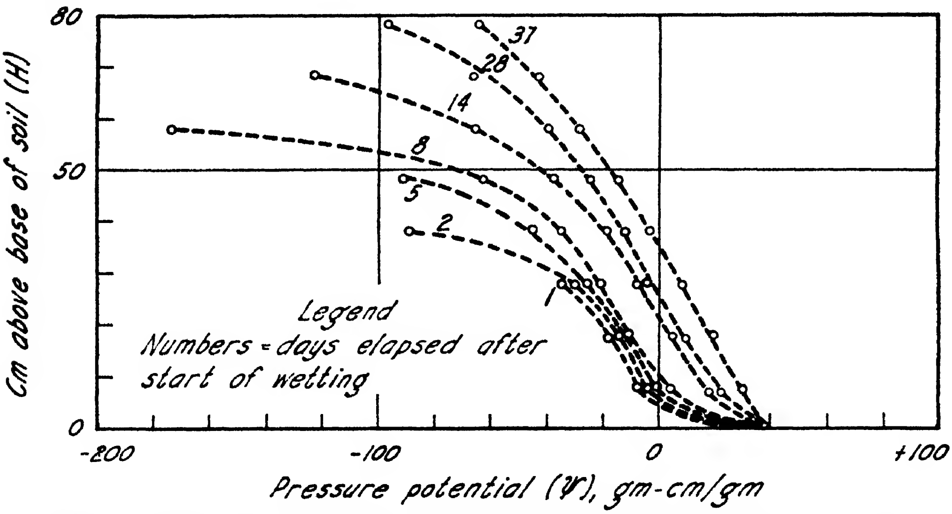


Fig. 4.—Distribution of pressure potential with the elevation above the base of the soil, $H = f(\psi)$ for a column of Yolo clay wetting.

log $A = f$ (log t) may be written in the form of an equation of a straight line,

$$\log A = -c\,(\log t) + \log K$$

or

$$A = kt^{-c}$$

where A is the rate of water uptake, t is the cumulative uptake time, and K and c are constants; c representing the slope or the rate of change of log A with log t. The data for all the soil columns investigated conform to

the first equation, with the exception that the value of c changed abruptly before the surface of the soil at the top of the columns had become obviously wet. Referring to figure 3, the initial stage of advance of the wetting front includes on the time scale, from inception of wetting to a log t in days of approximately 0.8 or an elapsed time of 6.3 days. During this time interval the wetting front advanced through air-dry soil of relatively constant apparent density and packing arrangement from near

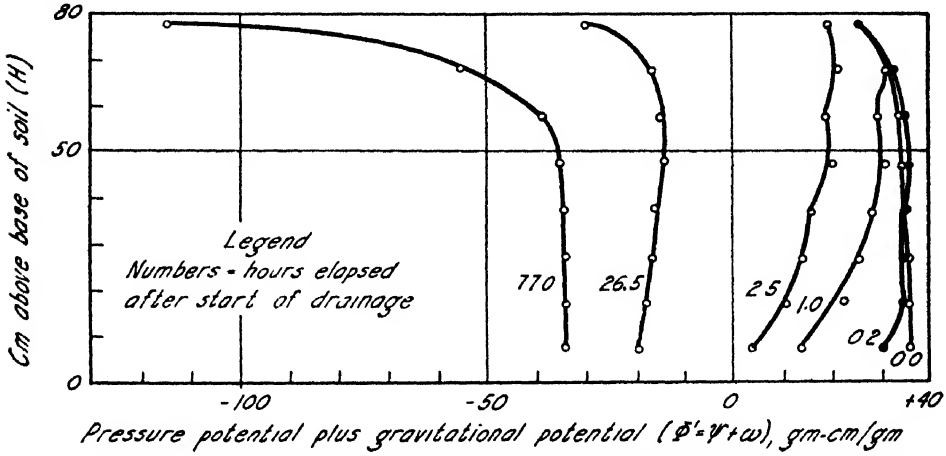


Fig. 5.—Distribution of the sum of the pressure and gravitational potentials with the elevation above the base of the soil, $H = f(\Phi')$ for a column of Yolo fine sandy loam drying.

the base of the soil column to within 1 or 2 mm of the surface. The 1 or 2 mm of soil at the top of the soil column was loosely packed and of lower apparent density than the remainder of the soil. Although the mulch was included in the length of the soil column, it is proposed that the contact between the mulch and the lower soil represented an irregular interface of discontinuity which was responsible for the change in the characteristic rate of water uptake.

A family of curves for Yolo clay representing the pressure potential distribution with elevation above the base of the soil at various cumulative wetting times is shown in figure 4, which, with the possible exception of the sands, is qualitatively characteristic of all the soils investigated during the wetting process. The potentials changed too rapidly in sands to be measured accurately.

Pressure Potential as a Function of Time During Drainage.—After upward flow in the soil columns had attained a steady state during the wetting process, the columns were drained by removing the water supply, and by maintaining a water-air interface at their bases. During drainage the change of pressure potential with time at various elevations

in the soil columns was recorded. The rate of change of pressure potential varied with texture, being greatest for sand and least for clay. The general character of the drainage curves, however, was similar for all the soils; therefore, data are presented for one soil only.

Figures 5 and 6 represent pressure potentials during drainage of a 3-foot column of Yolo fine sandy loam which had come to a steady state

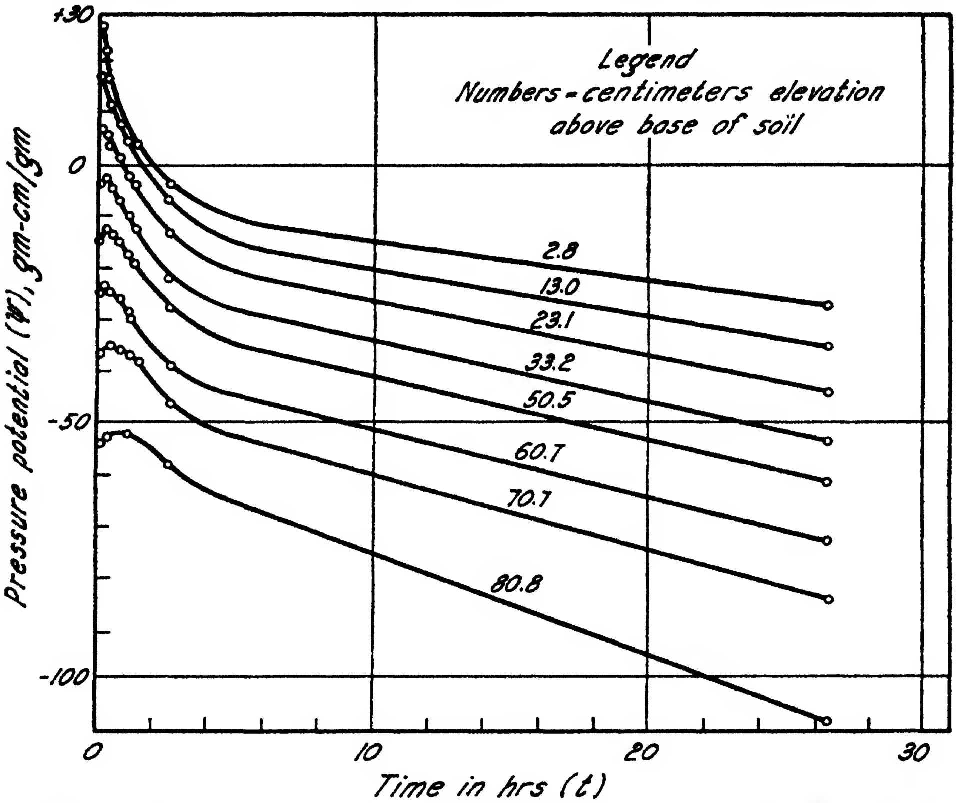


Fig. 6.—Distribution of the pressure potential at various elevations in the soil column with the time after drainage began, $\psi = f(t)$, in a column of Yolo fine sandy loam. Note the increase in pressure potentials in the unsaturated region of the column immediately after drainage began.

by wetting with a water pressure of 42 grams per square centimeter at the base of the soil column. The time when drainage began was arbitrarily taken as zero time, $t = 0$.

Figure 5 shows drainage curves for a column of Yolo fine sandy loam. For convenience in plotting, H is plotted as a function of Φ' which, however, should be considered the dependent variable, with the relation of Φ' to H expressed by the equation

$$\psi + \omega = \Phi' = f(H),$$

where H is the elevation above the base of the soil column in cm; ψ is the pressure potential in gm–cm/gm; ω is the gravitational potential in gm–cm/gm ($\omega = 0$ is arbitrarily taken at the elevation at which $\psi = 0$ when $t = 0$).

The total potential gradient had become approximately zero in the main body of the Yolo fine sandy loam column 60 hours after drainage began. This adjustment period required 24 hours and 96 hours in the Yolo sand and Yolo clay respectively.

Increases in pressure potentials immediately after drainage began were noted in all the cans at tensiometer positions above the water table (fig. 6). These increases indicated increases in the moisture density. Since even the highest tensiometer at an elevation of 80.8 cm above the base of the soil showed an increase in pressure potential, the water necessary must have been supplied from below.

A simple illustration taken from the case of a fully saturated single pore may explain the apparent anomaly of increasing pressure in the upper portion of a soil column induced by a sudden reduction in pressure at the base of the column. Figure 7 represents a capillary tube, the lower end of which had been dipped in water.

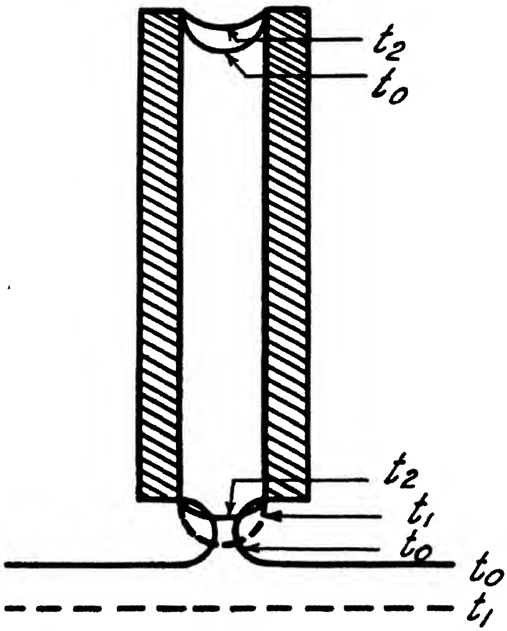
At time t_0 the water level has been lowered sufficiently to induce a temporarily reduced pressure potential about the lower tube opening, but at the same time to maintain a water connection between the free flat water surface and the capillary water in the tube. The pressure potential at the top of the capillary tube is ψ_0. Time t_0 represents the steady state condition in the soil columns at a position just above the surface of zero pressure potential.

At time t_1 the water level has been suddenly lowered. The water thread connecting the free flat water surface and the capillary water has broken. The water meniscus at the bottom of the capillary tube is convex to the air and the pressure immediately above the meniscus has increased from negative to positive.

At time t_2 the meniscus at the top of the capillary tube, which at t_0 was in equilibrium with a negative pressure at the base of the tube, has increased in radius of curvature to effect pressure equilibrium with the increased pressure at the base of the tube. An upward flow of water has taken place, and pressure potentials have increased throughout the capillary tube.

The discussion of the capillary tube in figure 7 deals with a fully saturated pore; whereas this experiment deals with a porous medium which is not saturated throughout its length, but is a three-phase system containing solid, liquid, and air. The energy involved, however, in the ad-

justment of water by capillarity in an unsaturated soil is due to the forces of gravity, adhesion, and cohesion, the same as are responsible for the movement of water in figure 7. The pressure potential changes induced by drainage of a soil column under the conditions of this experiment may be appropriately discussed by analogy to the capillary tube.



Time	Pressure in water under meniscus	
	Lower end	Upper end
t_0	−	−
t_1	+	
t_2	less +	less −

Fig. 7.—Diagrammatic illustration of the menisci and their changes in curvature during the withdrawal of a capillary tube from water.

The break in the continuity of the water system shown at time t_1 occurs in the large pores in the soil columns; the smaller moisture films in the soil which are capable of greater curvature remain continuous. Permeability in the moisture films of low curvature is relatively high; the pressure increase at the point of rupture of the large films postulated at a time corresponding to t_1 would result in an upward flow of water causing a temporary increase in pressure potentials.

General laboratory experiments on the distribution of water in soil over a water table have been conducted by setting soil tubes filled with

soil in vessels of water (*14, 15, 23*). In this type of experiment the soil rests on a porus support near the bottom of the soil tube and is in contact with water at atmospheric pressure. The elevation of the water in the outer vessel is kept constant, and it is assumed in these cases that the water table in the soil is at this elevation. After a duration of time, assumed to be sufficient for the establishment of a steady moisture distribution in the soil columns, the tubes are removed from the vessels of water and are sampled for moisture content. Such laboratory experiments have generally shown the highest moisture content to be some distance above the original water table. This distribution has been so universally observed in experiments that the experimental results have been interpreted as representing the actual moisture distribution in an undisturbed soil over a water table.

It should be observed here that removing the soil tubes from the vessels of water is not essentially different from the drainage technique reported in this paper. Consequently, the changes in pressure potentials during drainage which were observed in the experiment reported herein must have also taken place in the experiments cited above. These pressure potential changes (fig. 6) indicate that an upward flow of water takes place immediately after drainage begins, which flow may cause the highest moisture content, at the time the column is sampled, to occur at some distance above the original water table.

Effect of Temperature Variation on Pressure Potentials and Rate of Water Uptake.—The experiment was designed to study soil-moisture movement at constant temperature. Temperature variation in the soil was held within a range of 0.2° C except during four short periods during which a high positive correlation between increasing or decreasing temperature and certain observed features in the behavior of the soil-moisture system permits a statement of the qualitative effect of temperature variation on these features.

A drop in temperature always resulted in an increased rate of water intake and in lower pressure potentials throughout the columns in all the soils. A rise in temperature had the reverse effects. A drop in temperature of 1° C in one hour lowered the water table 3 cm in the Yolo sand and 12 cm in the Yolo clay with intermediate amounts for soils of intermediate texture.

In the soil columns at steady state, with temperature constant, the soil-moisture system is in delicate balance, water flows through the column at a constant rate, and the pressure potentials throughout the soil mass are steady and dependent upon the permeability of the soil and the velocity of flow. The moisture gradient is fixed by the pressure potential

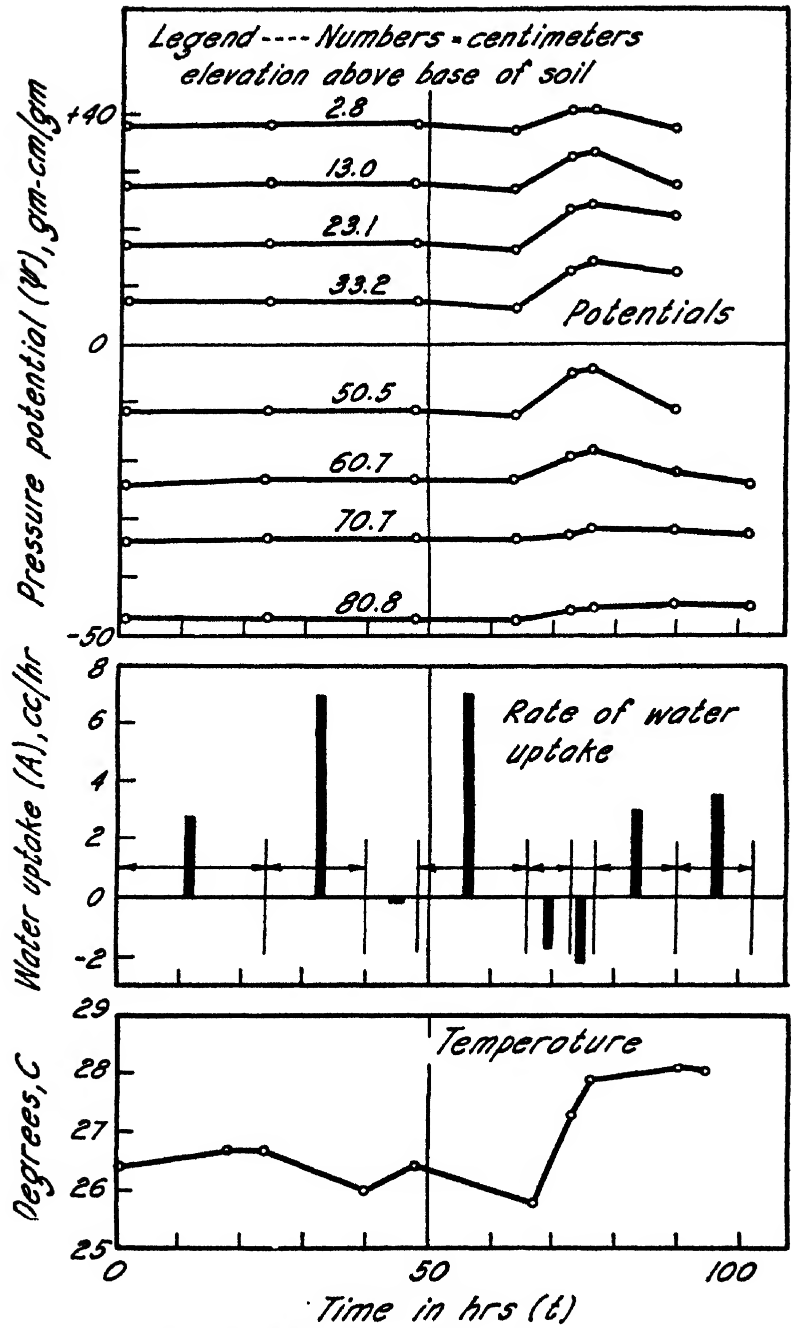


Fig. 8.—The effect of changes in temperature on pressure potentials and rate of water uptake in a column of Yolo fine sandy loam.

gradient, the displacement of which initiates adjustments in the soil-moisture density in the direction of re-establishment of the steady-state gradient.

Surface tension decreases and pressure potential in unsaturated soil increases with increasing temperature. Therefore, an unsaturated soil at a given potential holds less water at a higher than at a lower temperature. After a rise in temperature, water drains downward through the soil, gradually reducing the pressure potentials in the upper portion of the soil column and increasing still further the pressure potentials in the lower portion. During this phase of readjustment, temporary water tables were recorded up to 15 cm higher than the pressure head of the supply line. With decrease in temperature, the cycle described above is reversed; the pressure potentials become more negative, the rate of water uptake increases, and the water table drops.

The magnitude of the potential variations and changes in rate of water uptake with changes in temperature depend upon the rate of temperature change, the permeability of the soil, and the relation of pressure potential to moisture content. If the change in temperature is sufficiently slow or if the soil permeability is sufficiently high, redistribution of water in the soil may take place with sufficient rapidity to maintain relatively constant potentials, and the only obvious major deviation from steady state is the rate of water uptake.

The performance of a column of Yolo fine sandy loam during a period of temperature variation is shown graphically in figure 8. At zero time the pressure potentials in the soil column were at steady state with a rate of water uptake 3.9 cubic centimeters per hour, and a temperature (T) of 26.4° C. The graph may be divided into several time periods for the purpose of discussion:

1. $t = 0$ hours to $t = 24$ hours.
   - A. $\Delta T/\Delta t = +\ 0.013°$ C per hour.
   - B. Small increase in pressure potentials.
   - C. Decrease in rate of water uptake.
2. $t = 24$ hours to $t = 40$ hours.
   - A. $\Delta T/\Delta t = -\ 0.044°$ C per hour.
   - B. No appreciable change in pressure potentials.
   - C. Great increase in rate of water uptake.
3. $t = 40$ hours to $t = 48$ hours.
   - A. $\Delta T/\Delta t = +\ 0.05°$ C per hour.
   - B. No significant change in pressure potentials.
   - C. Rate of water uptake decreased to a negative value.

4. $t = 48$ hours to $t = 67$ hours.
   A. $\Delta T/\Delta t = -0.032°$ C per hour.
   B. Slight decrease in pressure potentials.
   C. Great increase in rate of water uptake.

5. $t = 67$ hours to $t = 76$ hours.
   A. $\Delta T/\Delta t = +0.23°$ C per hour.
   B. Increase in pressure potentials of 2 gm–cm/gm at 81 cm above the base of the soil column, and increasing progressively to more than 10 gm–cm/gm at the water table. Water flowed downward through the soil and out of the can via the supply line. Greater potentials would have been registered if water had not been lost from the system.
   C. The rate of water uptake became negative. The negative absorption rates shown on the graph do not represent the total amount of water drained out of the soil column. An undetermined amount of water was lost through the vent tube in the constant-level supply reservoir.

6. $t = 76$ hours to $t = 95$ hours.
   A. Temperature changing very slowly and approaching 28.2° C.
   B. Pressure potentials rapidly decreasing toward establishment of of a steady-state gradient approximately equivalent to that at $t = 0$.
   C. Rate of water uptake approaching the rate at the previous steady state.

It is evident that temperature variations may vitiate the accuracy of many types of soil-moisture studies. Fluctuations in soil moisture contents and water tables in field studies may be erroneously attributed to causes other than temperature unless the variations in temperature and their attendant effects are known (*25–27*). The quantitative evaluation of the various effects that temperature variations may have on the soil moisture system would constitute a major problem.

Pressure Potential and Moisture Distribution at Steady State.—Pressure potential and moisture content data in soil columns at steady state are tabulated in table 3, and are represented graphically in figures 9–13. The moisture samples were approximately 10-gram core samples taken with a thin-walled, polished, aluminum tube ½ inch in diameter which was thrust horizontally into the soil column through holes in the side of the soil can. The soil sample was quickly transferred to a weighing bottle by pushing the core out of the sampling tube with a tight-fitting plunger. A soil column was sampled, taking 16 samples, in about 3 minutes.

The tube method of sampling was satisfactory for moisture contents up to approximately 85 to 90 per cent of saturation, but beyond this moisture range very erratic results were secured. Great limitations were imposed on the possible methods for taking samples by the design of the

TABLE 3*

MOISTURE CONTENT AND PRESSURE POTENTIAL; EXPERIMENTAL VALUES FOR YOLO LIGHT CLAY, CAN NO. 2†

Elevation above water table	Wetting		Drying	
	Moisture content, P_w (oven-dry basis)	Pressure potential, ψ	Moisture content, P_w (oven-dry basis)	Pressure potential, ψ
cm	*per cent*	*gm–cm/gm*‡	*per cent*	*gm–cm/gm*
3.5	33.8	− 3.7		− 11.0
8.7	36.0		34.92	
13.9	34.1	− 15.1		− 23.0
19.0	35.8		33.53	
24.2	30.3	− 26.5		− 34.3
29.0	33.8		30.58	
34.1	30.0	− 39.6		− 44.1
39.1	28.7		28.60	
44.2	28.2	− 56.2		− 62.8
49.4	28.0		27.55	
54.6	26.8	− 75.8		− 84.5
59.6	26.4		26.49	
64.6	25.1	− 96.4		−104.7
69.0	24.9		24.50	
74.6	23.8	−134.9		−135.0
79.7	23.4		23.15	
84.8	22.3	−198.8		−200.0
90.0	21.2		21.31	
95.1	19.8	−359.5		−377.6
100.1		−599.0	18.54	−610.0
105.1	{14.4 {14.7			

* Plotted in figure 11

† Owing to the limitations of space in this paper, tabular experimental data on the distribution of pressure potentials and moisture is given for one soil column only.

‡ Gm–cm/gm and gm/gm are used throughout this paper as units of work and of force respectively. These values may be multiplied by 980 to obtain dynes and ergs.

soil cans, and the necessity for a minimum disturbance of the soil and alteration of the cross section of the column. The tube method was used for all sampling.

The $P_w = f(H)$ curves (figs. 9–13) were plotted from the experimental data from the lower moisture contents up to 85 to 90 per cent of saturation, and projected from this point to saturation. Experimental values for moisture contents were disregarded in the wet portion of the curve. The zone of saturation was assumed to extend from the base of the soil through the region of positive pressure potentials, and to the

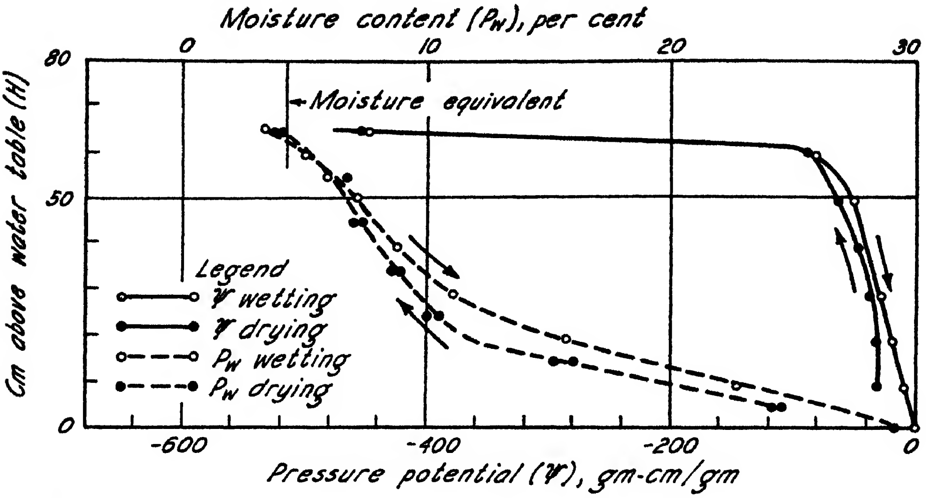


Fig. 9.—Curves of $P_w = f(H)$ and $\psi = F(H)$ for a column of Oakley sand at steady-state wetting and after drainage. For convenience in representation, P_w and ψ are plotted as abscissas.

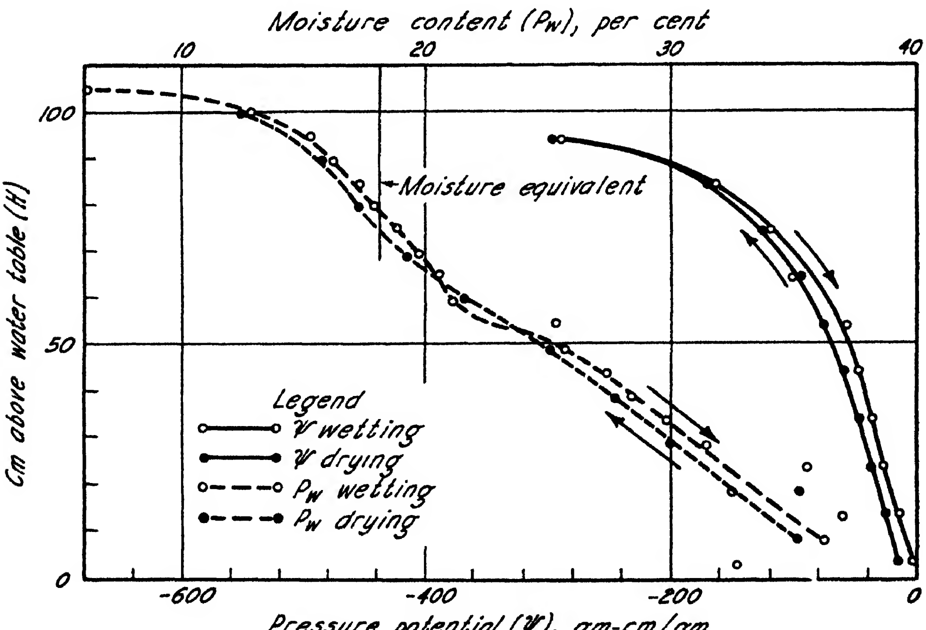


Fig. 10.—Curves of $P_w = f(H)$ and $\psi = F(H)$ for a column of Yolo fine sandy loam at steady-state wetting and after drainage. For convenience in representation, P_w and ψ are plotted as abscissas.

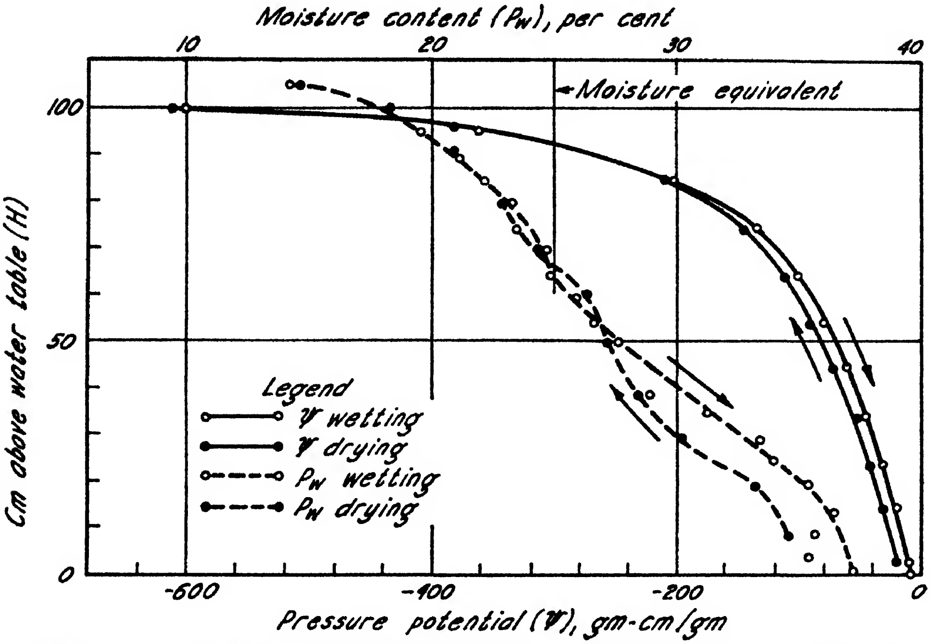


Fig. 11.—Curves of $P_w = f(H)$ and $\psi = F(H)$ for a column of Yolo light clay at steady-state wetting and after drainage. For convenience in representation, P_w and ψ are plotted as abscissas.

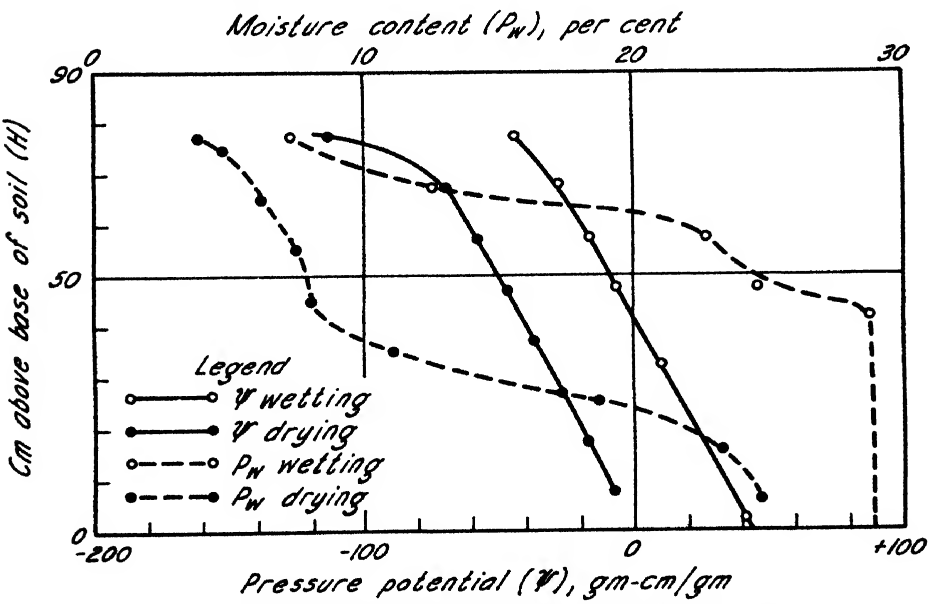


Fig. 12.—Curves of $P_w = f(H)$ and $\psi = F(H)$ for a column of Yolo sand at steady-state wetting and after drainage. For convenience in representation, P_w and ψ are plotted as abscissas.

elevation of zero pressure potential. The moisture content of the soil at saturation was calculated from the apparent density of the soil, assuming a real density of 2.61.

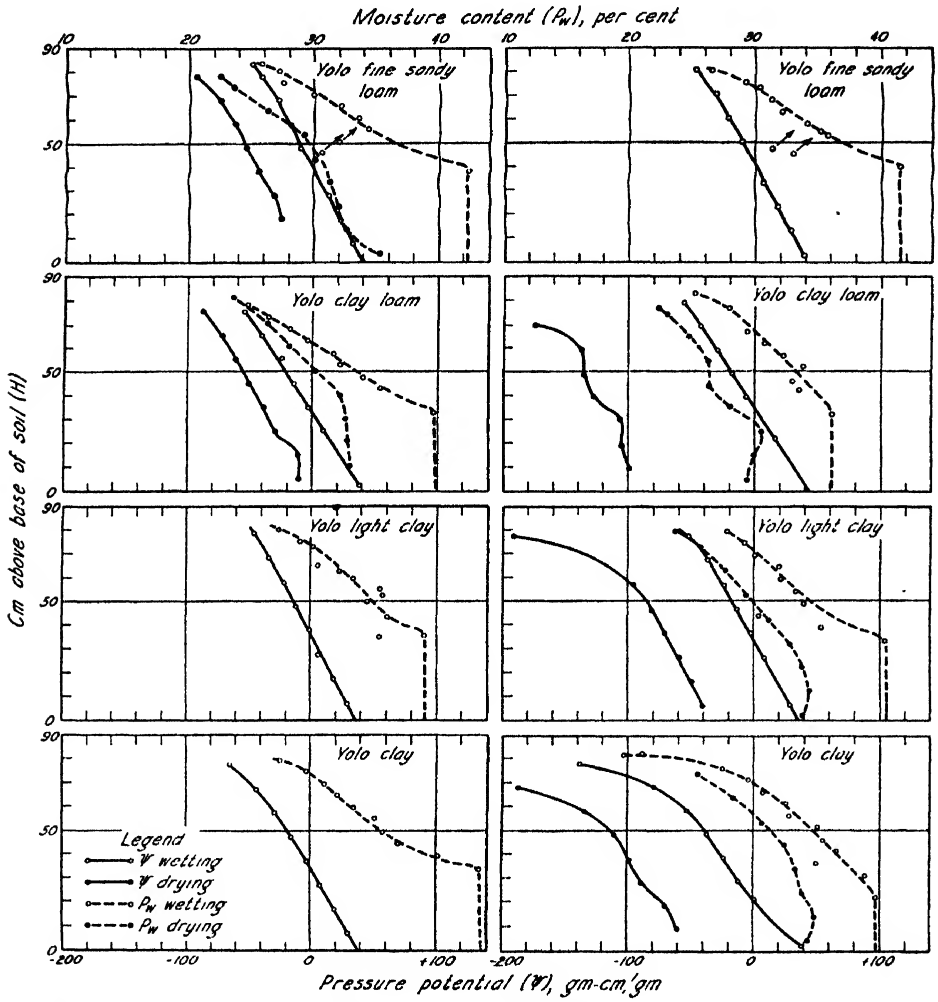


Fig. 13.—Curves of $P_w = f(H)$ and $\psi = F(H)$ for columns of soil at steady-state wetting and after drainage. For convenience in representation, P_w and ψ are plotted as abscissas.

ANALYSIS OF THE PRIMARY EXPERIMENTAL DATA

The Relation between Pressure Potential and Water Content of Soils at 29° C.—The relation between pressure potential, ψ, and moisture content, P_w, in each soil column is represented graphically in curves (figs. 14–18) of $\psi = f(P_w)$. These curves were developed for the soils at a

steady state during the wetting process, and after drainage, from the primary moisture content and pressure potential data as expressed in curves of $P_w = f(H)$ and $\psi = F(H)$.

Differences in vapor pressure and pressure potentials for the same medium at the same moisture content, the magnitude of which depends

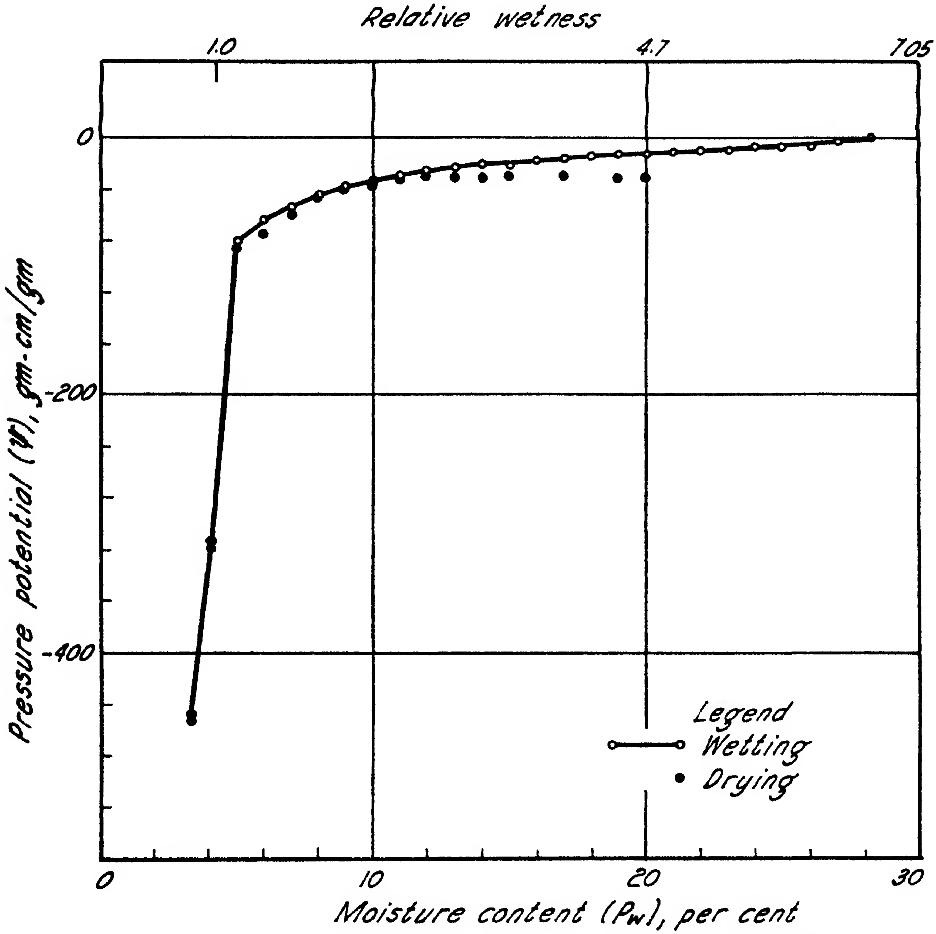


Fig. 14.—Curve of $\psi = f(P_w)$ for Oakley sand wetting and drying.

upon whether the medium is wetting or drying, have often been reported. This phenomenon has been referred to hysteresis effects (*11, 12, 21*), and has been reported in a wide variety of media (*32*) in which capillarity is active in the distribution and retention of liquids. Hysteresis has been attributed by the early workers to the alteration of the contact angle between solid and liquid due to adsorbed air on the solid. Adam (*1*) attributes the alteration of the contact angle to the frictional resistance

between liquid and solid. Smith (*29, 30*) and his associates working with an "ideal soil" attribute maximum, minimum, and intermediate capillary rise to the cyclic alteration in pore cross section incident to any type of packing of spheres. Hysteresis is generally reported for wetting and

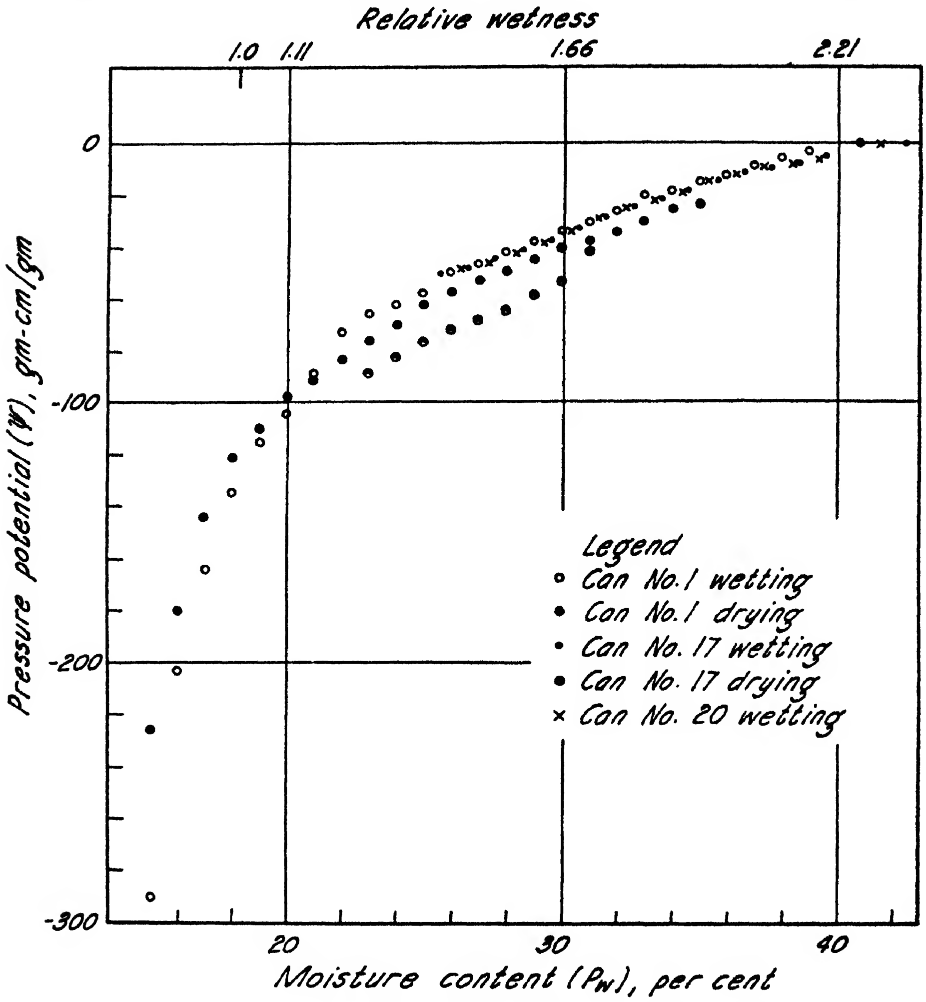


Fig. 15.—Curve of $\psi = f(P_w)$ for Yolo fine sandy loam wetting and drying.

drying clays, and for colloidal separates of clays dried or wetted in evacuated desiccators over osmotic solutions.

In the procedure followed in this experiment, drainage of the soil columns was accompanied by flow of water from the tensiometer cup to the soil, and by settlement and attendant increase in apparent density of the soil. A lag in pressure equilibrium between the tensiometer cup

and the soil would have indicated an apparent hysteresis, but opposite in sign to that observed. At a given moisture content and increased apparent density, the negative radius of curvature would increase and the vapor pressure and the pressure potential should increase. Settlement

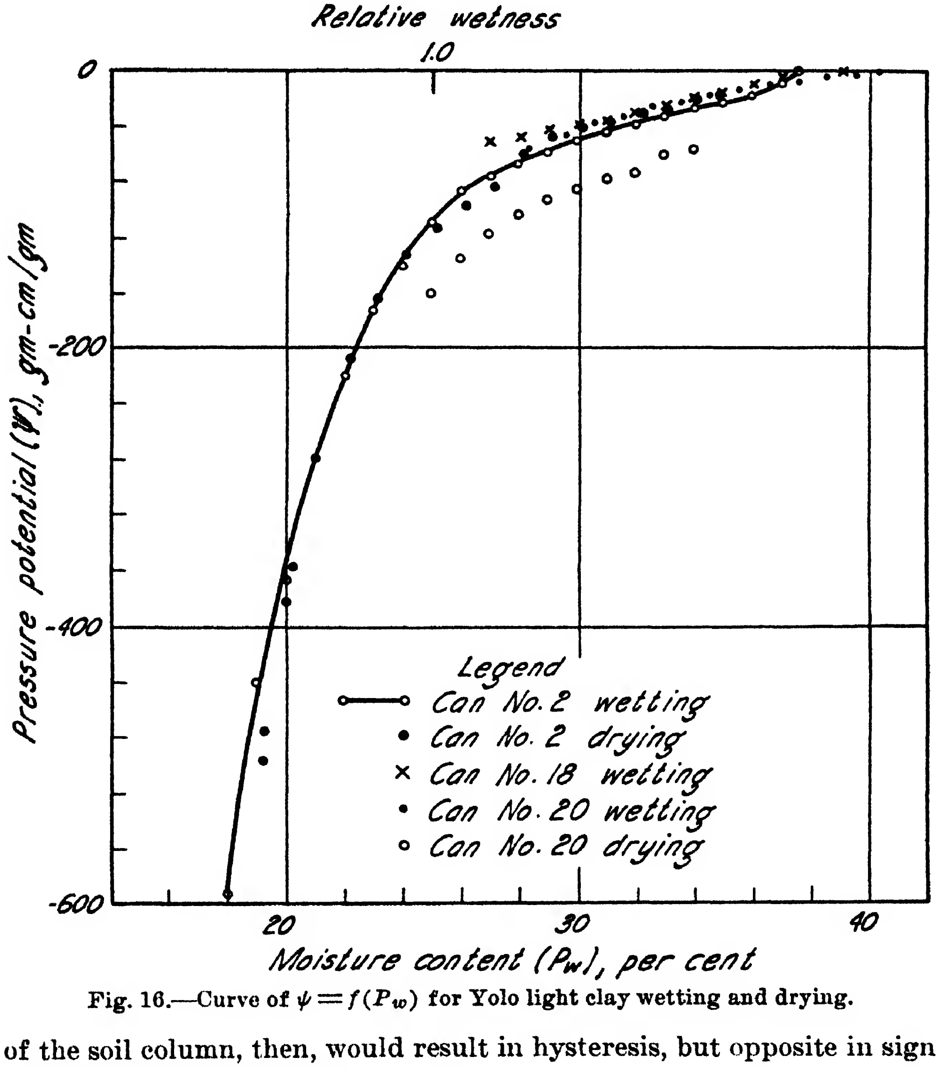


Fig. 16.—Curve of $\psi = f(P_w)$ for Yolo light clay wetting and drying.

of the soil column, then, would result in hysteresis, but opposite in sign to the hysteresis observed.

The differences between the wetting and drying pressure potential curves extend almost to saturation, the range of the negative radii of curvature as calculated from the pressure potentials and surface tension being from 14 to 50 microns. Within this range of moisture content, the solid particles must be entirely bathed with water, and probably no

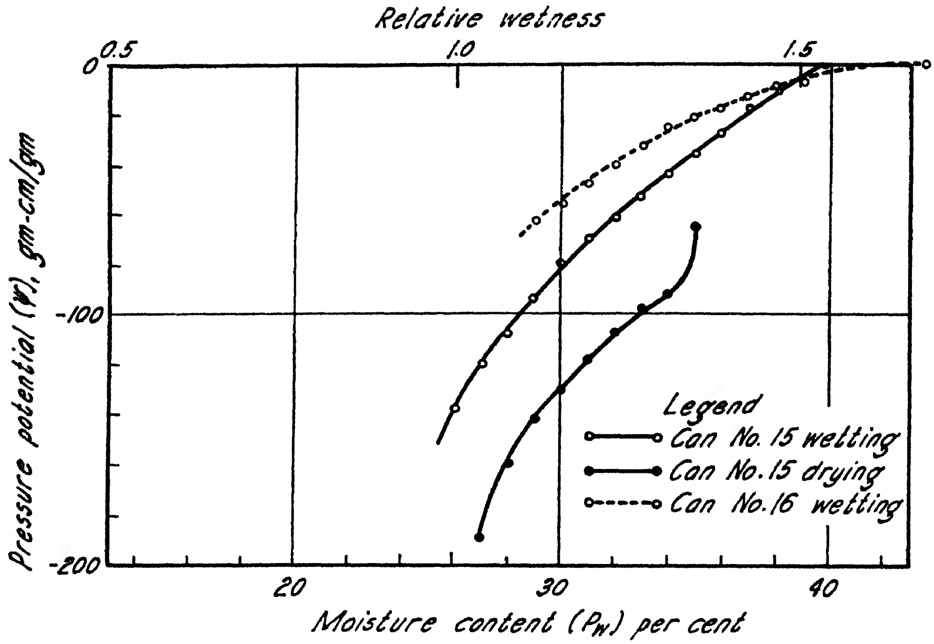


Fig. 17.—Curve of $\psi = f(P_w)$ for Yolo clay loam wetting and drying.

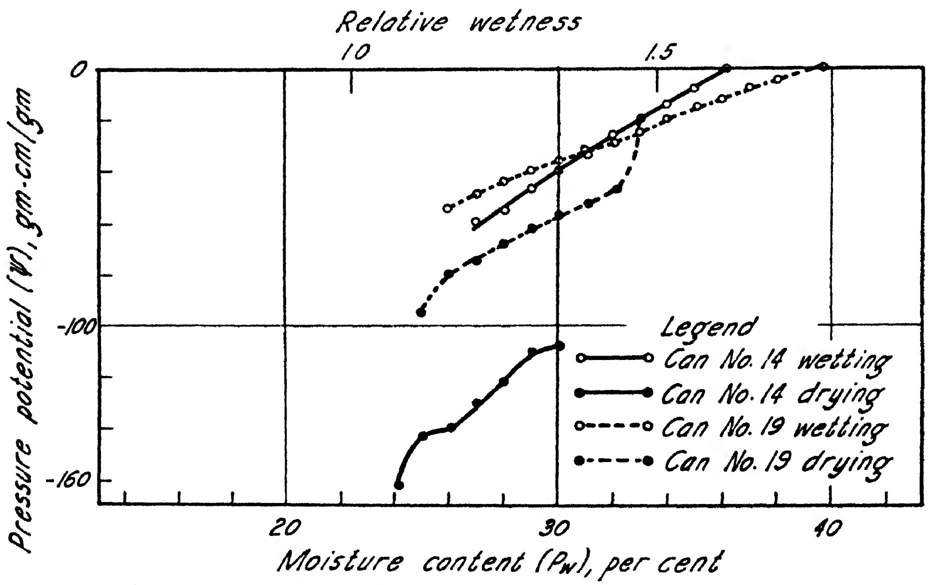


Fig. 18.—Curve of $\psi = f(P_w)$ for Yolo clay wetting and drying.

solid-air interface exists. Under such conditions, adsorbed air on the surface of the solid particles should play little or no part in the curvature of the water-air interface and resulting pressure potentials.

This investigation contributes no positive evidence as to the causative factors in hysteresis; however, the following enumeration of the conditions under which hysteresis was observed may contribute by limiting the field of conjecture as to these factors:

1. At a given negative pressure potential, soils held more water on drying than on wetting.

2. The calculated negative radii of curvature of menisci, within which range the principal hysteresis was observed, were 14 to 50 microns. Hysteresis may persist at radii much less than 14 microns, but under the experimental conditions imposed, drying did not proceed readily below that radius.

3. Soils settled and increased in apparent density as drainage progressed. If an increase in apparent density is the only significant structural change involved in shrinkage, then settlement would result in a higher rather than a lower pressure potential for a given moisture content.

4. As the soils dried, water flowed from the tensiometer cup to the soil. A lag in pressure equilibrium between the cup and the soil would result in a higher, rather than a lower, pressure potential for a given moisture content.

5. The magnitude of hysteresis increased as the range between the wetting moisture content and the drying moisture content increased, as indicated by the following data:

	Can No.	Per cent loss of water during drainage	Approximate hysteresis in gm—cm/gm
Yolo fine sandy loam	1	1	5 to 10
	17	5 to 12	10 to 20
Yolo light clay	2	2	+8 to —8
	20	4 to 6	40
Yolo loam	19	1 to 7	20
	14	5 to 8	70

It has been suggested that tensiometers installed in the soil would be a practicable means of charting the course of the soil moisture status through the relation of pressure potential to moisture content. The above data, however insufficient, indicate that the pressure potential may be not only dual-valued in terms of water content, but that the magnitude of the drying potential may depend upon the range through which the soil dried.

The Permeability of Soils to Water.—In slow motion such as that of water through soils, the velocity of flow, V, may be expressed as a product of the force acting to drive the water through the soil, and of a conductivity function, or the permeability of the soil to water, K. The mathematical expression of flow is given in the equation (*4*),

$$V = KF = -K\nabla(\psi + \omega + \lambda) = -K\nabla\Phi$$

where V represents the mean velocity of flow; F, proportional to V, is the total force per unit of mass acting to drive water through the soil

TABLE 4

Velocity of Flow of Water in the Soil Columns at Steady State

Soil type and can number		Distance from the water table to the surface of the soil column, centimeters	Depth of water transported to the surface	
			Centimeters×10⁶ per second, V	Centimeters per day
Oakley sand, 3		105	0.23	0 02
Yolo sand, 13		42	0 52	0 04
Yolo fine sandy loam	1	105	0 37	0 03
	17	46	5 30	0 46
	20	42	5 60	0 48
Yolo clay loam	14	51	3 50	0 30
	19	50	5 50	0.47
Yolo light clay	2	105	0 87	0 08
	18	46	6.50	0.56
	20	43	7.10	0 61
Yolo clay	15	60	2 90	0 25
	16	50	5.30	0.46

and is composed of the gradients of pressure potential, ψ; the gravitational potential, ω; and the osmotic potential, λ. The permeability, K, is constant and independent of the rate of flow and the total potential gradient, $\nabla\Phi$.

In applying the general formula to the calculation of permeabilities in the soil columns, V (table 4) is expressed in centimeters per second, $F = -\nabla\Phi$ is expressed in grams per gram, and K has the dimensions of time. The gravitational potential gradient, $\nabla\psi$, is assumed to be constant and equal to one gram per gram. The osmotic potential, λ, is assumed to be constant and the osmotic potential gradient, $\nabla\lambda$, is therefore equal to zero. According to the above assumptions, and under the condition of this experiment in which all movement other than vertically upward or

downward was eliminated, the total potential gradient in grams per gram may be written

$$\nabla\Phi = \nabla\psi + 1,$$

and since the pressure potential gradient at steady state was negative upwards

$$K = \frac{V}{-(\nabla\psi + 1)}.$$

The pressure potential gradient was evaluated at any desired points in the soil column by drawing tangents to the $\psi = f(H)$ curve for that column and determining their slope.

In this discussion of the permeability of soil to water, it is not intended to introduce new terms. In the interest of clarity, however, it is necessary to restate the definition of terms that will be frequently used:

Saturated permeability refers to the permeability of the soil when the soil is saturated with water. Saturated soil is a two-phase system, solid and liquid.

Unsaturated permeability is the permeability of the soil when it is unsaturated. Unsaturated soil is a three-phase system, solid, liquid, and gas. Unsaturated permeability is based on the flow of water through the soil in the vapor phase, or in the vapor and liquid phases.

Capillary permeability refers to liquid flow in unsaturated soil.

Vapor permeability refers to vapor flow in unsaturated soil.

The configuration of the moisture system, in soil at various degrees of unsaturation, has been amply discussed elsewhere in the literature; here, it is sufficient to say that vapor flow will take place in unsaturated soil at any total potential gradient other than zero. Capillary flow can take place only at those soil-moisture contents at which the water films are so connected as to allow liquid water to flow through water films from one position in the soil to another. *Continuous water films* are those which permit water, in the liquid phase, to flow through water films from one position in the soil to another. Water films are termed discontinuous when water in the liquid phase cannot flow through water films from one position in the soil to another. Continuous water films must be connected, but discontinuous water films may also be connected, the sole criterion for continuity, as here used, is that of flow as described above. If the soil moisture films are continuous, capillary flow will take place at any total potential gradient other than zero. If the soil moisture films are discontinuous, capillary flow is zero (*18*).

Permeability as a Function of Pressure Potential.—Soil permeability is measured per unit of cross-sectional area of soil. Since capillary flow

takes place through moisture films, we would expect capillary permeability to increase with increasing effective cross section of moisture films, and hence with increasing moisture in the soil. In this study the moisture content has been expressed as a function of pressure potential, ψ, which is directly related to the vapor pressure of the soil moisture, and is an

TABLE 5*

WETTING AND DRYING POTENTIALS AND PERMEABILITY AS FUNCTIONS OF MOISTURE CONTENT; YOLO LIGHT CLAY, CAN No. 2

P_w	Wetting		Drying	
	ψ	$K† \times 10^6$	P_w	ψ
18	−592	0 012		
19	−440	0 022	19.2	−476
20	−366	0 041	20.2	−358
21	−278	0 064	21.2	−280
22	−224	0 091	22 2	−208
23	−172	0.133	23.2	−162
24	−140	0 215	24 2	−126
25	−108	0 398	25 2	−111
26	− 86	0.795	26 2	− 98
27	− 76	0 88	27 2	− 82
28	− 67	0 97	28 2	− 60
29	− 58	1 06	29 2	− 48
30	− 50	1 21	30 2	− 41
31	− 44	1.48	31 2	− 36
32	− 37	1 91	32 2	− 31
33	− 32	2 56	33 2	− 28
34	− 27	3 30	34 2	− 23
35	− 22	4.40	34 9	− 16
36	− 16	6 15		
37	− 8	8 60		
37 5	0	12 3		

* Owing to the limitations of space in this paper, tabular experimental data on the distribution of pressure potentials and moisture is given for one soil column only

† Permeability, K, was calculated from the velocity of flow, V, expressed in centimeters per second and the pressure potential gradient, $\nabla\psi$, expressed in grams per gram. The values for V were taken from table 4, and the values for $\nabla\psi$ were taken as the slope of the curve of $\psi = F(H)$ in figure 11.

especially useful function of the soil moisture density because it is also an index of its configuration. Accordingly, unsaturated permeability will be investigated as a function of ψ (table 5 and figs. 19, 20).

Permeability is maximum at, or near, a pressure potential of zero for all the soils covered by this experiment. The permeability remains constant from zero pressure potential down to pressure potentials of — 10 to — 40. Saturation persists for some distance above the plane of zero-pressure potential, but this distance could not be determined by the method used for taking moisture samples. This height was probably from 1 to 3 centimeters above the plane of zero-pressure potential, and

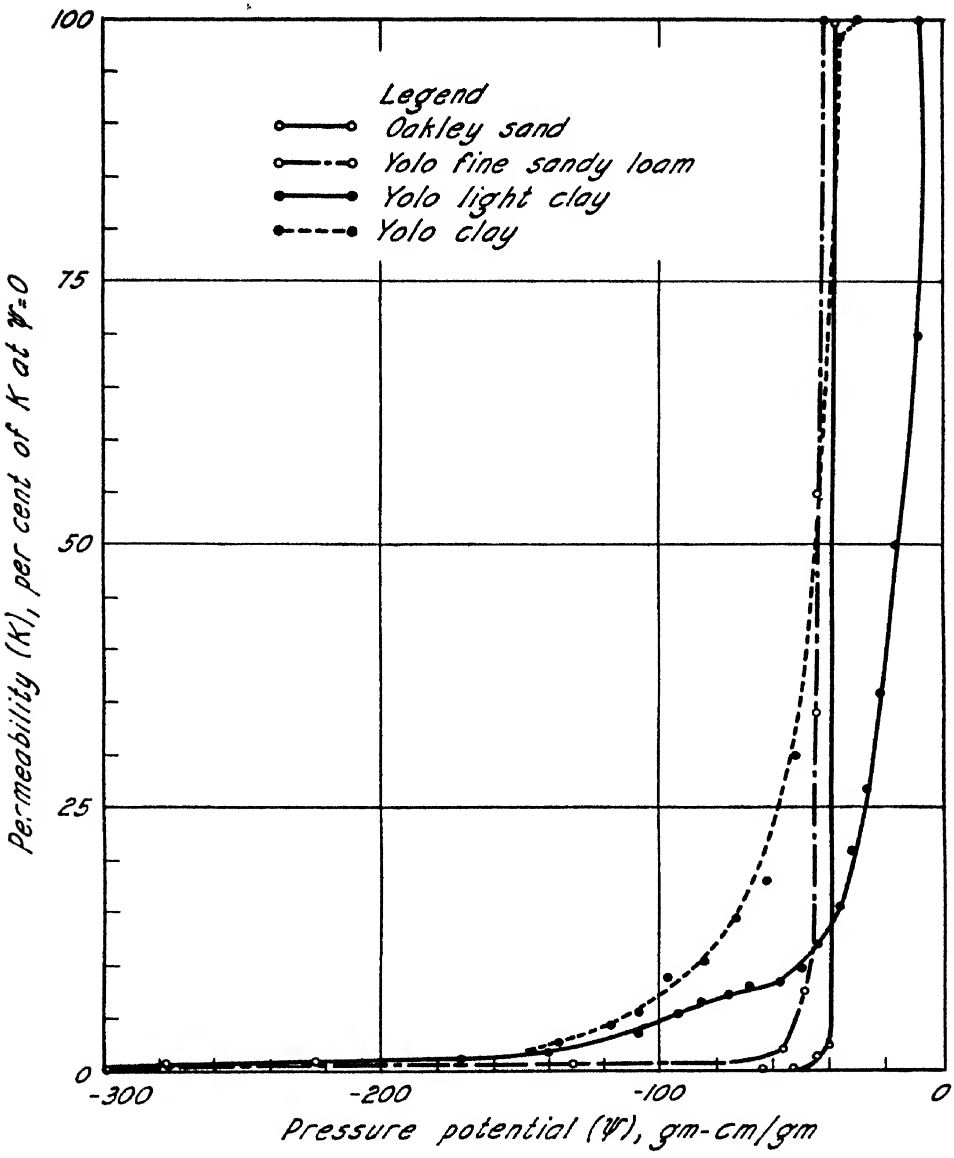


Fig. 19.—Permeability as a function of pressure potential, $K = f(\psi)$ with permeability expressed as a percentage of the permeability at a pressure potential of zero.

was entirely too small to account for the high permeability extending 20 to 30 centimeters above the piezometric surface.

Permeability of the Yolo clay was less in the range of positive pressure than that at pressure potentials of zero to —40 gm–cm/gm. Under certain conditions differential swelling of soil colloids effected by wetting at

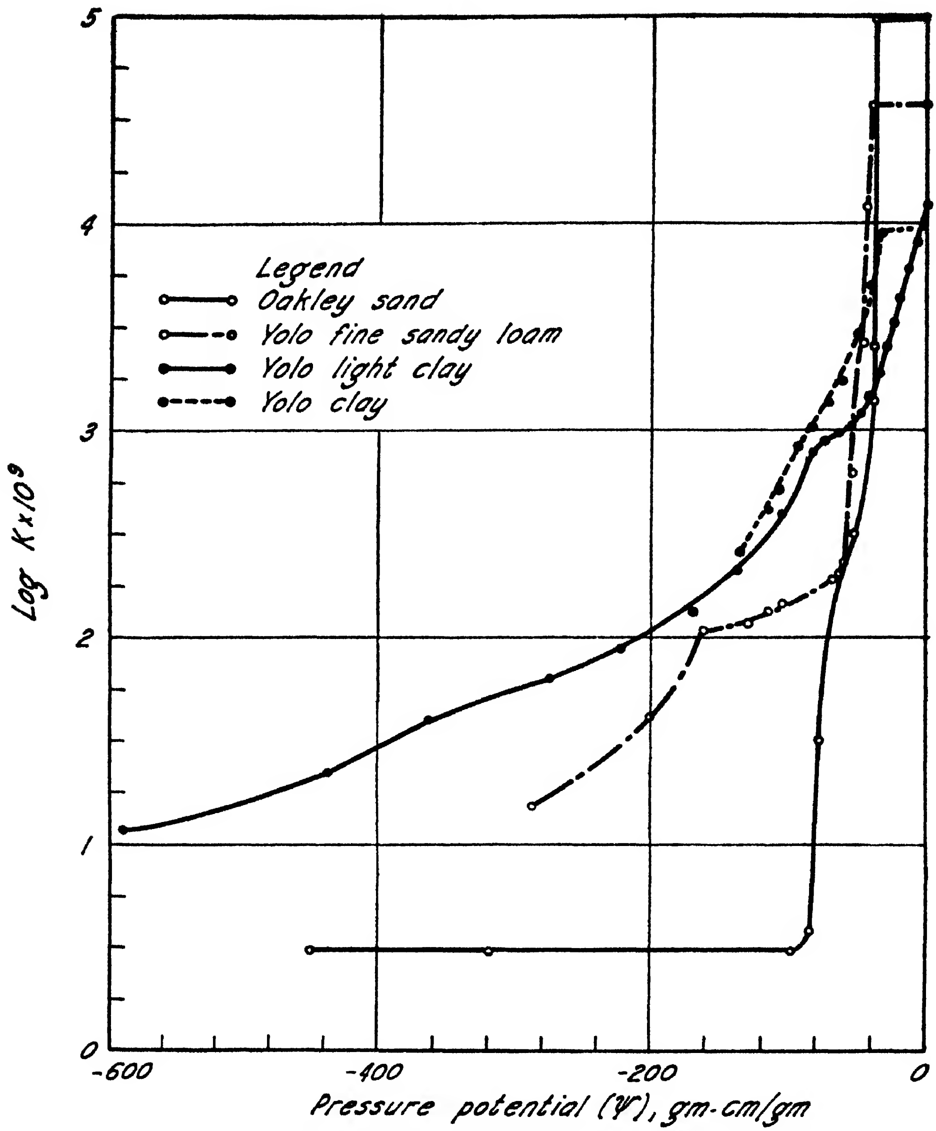


Fig. 20.—The logarithm of the permeability times 10^9 as a function of the pressure potential, log $(K \times 10^9) = f(\psi)$, plotted for four soils. $K = -\frac{V}{\nabla\Phi}$, in which V is expressed in cubic centimeters per second, and $\nabla\Phi$ in grams per gram.

different pressure potentials may result in a greater permeability above the piezometric surface than that in the range of positive pressure. Yolo clay swells on wetting, but no change in the over-all volume of the soil was detected in the cans. In such swelling the solid particle with its absorbed water may act as a larger solid particle, as far as the permeability

of the swollen soil is concerned. This would result in a greater effective volume of solid and lesser effective volume of pore space per unit volume of soil, and likewise, a lower total effective pore area and fewer continuous pores per unit of cross-sectional area of the soil column. If the soil colloids swell in proportion to the pressure potential at which they are wetted, the total pore space effective in the conduction of water in the soil columns would decrease as this potential increased. Hence the per cent of effective pore space in the range of positive potentials would be less than in the range of negative potentials, and permeability may be greater for some distance above the piezometric surface than that obtained below this surface.

The Effect of Texture on Soil Permeability.—The effect of texture on the manner with which permeability changes with pressure potential is shown in figures 19 and 20. For the soils studied, saturated permeability increases with increasing coarseness of texture. In the range of capillary flow, the rate of change of permeability with pressure potential, $\partial K/\partial\psi$, increases with increasing coarseness of texture, such as to produce the reversal of permeabilities shown in figure 20. The soils arranged in permeability series are, at saturation:

$$\text{sand} > \text{fine sandy loam} > \text{light clay} > \text{clay}$$

and at $\psi = -100$

$$\text{sand} < \text{fine sandy loam} < \text{light clay} < \text{clay}.$$

Zero Capillary Permeability.—The evaluation of the soil moisture content, or the pressure potential in the soil at which capillary conductivity becomes zero, is of importance in the study of soil-moisture problems, such as: the distribution of water in the soil, the translocation of soluble salts, the maximum height of capillary rise, etc. Richards (*18*) states that the water in soils is no longer present in a continuous liquid phase, and capillary flow ceases at the point where capillary conductivity becomes zero. At field capacity or at the normal moisture capacity the capillary permeability of soils must be zero or approximately zero and any water translocation must take place in the vapor phase (*13*, *22*, *33*, *34*).

In this paper zero capillary permeability is discussed under the two criteria: the pressure potential of the wetting front, and the pressure potential at which $\partial K/\partial\psi = 0$.

The advance of the wetting front was observed in the soil columns through celluloid-covered holes in the soil cans. Core moisture samples were taken at the wetting front with a ¼-inch cork borer, care being taken to include no dry soil. The center of the core was about $^3/_{16}$-inch

below the wetting front. Since there was a moisture gradient in the soil column with the moisture density increasing with distance below the front, the samples may show a higher moisture content than that of the wetting front. The increase in the experimental value for moisture content due to a moisture gradient could not be determined because the gradient itself could not be evaluated. The moisture gradient, however, decreases with increasing height of rise; and if the effect of the moisture

TABLE 6

MOISTURE CONTENT AT WETTING FRONT FOR THREE YOLO SOILS

Fine sandy loam, can No. 17		Light clay, can No. 20		Clay, can No. 16	
Elevation of sample	P_w	Elevation of sample	P_w	Elevation of sample	P_w
cm	*per cent*	*cm*	*per cent*	*cm*	*per cent*
13.1	19.9	7.6	24.6	19 9	26.1
20 4	19.6	10 3	25 1	30.0	26.8
23 2	20.2	27 6	24 3	40 1	24 9
27 9	21.0	35.2	24.0	50.3	25.9
33 4	20.5	37 8	24 4	70 5	25 6
35.9	20.8	40 5	24.1		
40.6	21 6	50 5	25.9		
50.7	19.8				
70 9	20.6				
81.1	19.7				
Mean P_w (per cent) 20 4			24.6		25 9
ψ (gm–cm/gm)* −92			−120		−140

* ψ values taken from $\psi = f(P_w)$ curves.

gradient is appreciable, the moisture content of the wetting front samples should decrease from the bottom of the soil column to the top. No such relation was found; the wetting front samples showed a relatively uniform moisture content throughout the length of the soil columns. Table 6 lists wetting front moisture contents with the elevation of the front above the base of the soil columns.

Wetting front samples were not taken from the Oakley sand; but from the nature of its curve of $\psi = f(P_w)$ it is assumed to be at a pressure potential of approximately — 80 gm–cm/gm, and a moisture content of 5.0 per cent.

The following experimental observations, along with certain theoretical considerations, may aid in characterizing the wetting front:

1. Water advances in a front from wet to drier soil under the influence of capillarity (*14, 36*). Beyond the front, the soil remains apparently dry, and immediately at and behind the front, the soil is apparently

completely wetted. Macroscopically, there is a sharp line of demarcation between the obviously wet soil and the obviously dry soil.

2. The moisture content of the wetting front determined by sampling is constant, indicating a constant potential and a constant radius of curvature. The pressure potential of the wetting front ranged from — 80 gm–cm/gm for the Oakley sand to — 140 gm–cm/gm for the Yolo clay with intermediate values for soils of intermediate texture.

3. The moisture content of the dry soil immediately beyond the wetting front was not definitely known. The air-dry soil was in equilibrium with air at a relative humidity of approximately 42 when wetting began. As the first approximation we may assume a relative humidity of 50 at a distance of 1 mm beyond the wetting front making the pressure potential gradient across this region 1×10^7 gm/gm. These assumptions may be in appreciable error, but whatever logical values are assumed, the pressure potential gradient from the dry soil to the wet is obviously very great.

4. The existence of a great pressure potential gradient from the dry soil to the wetting front should insure the maintenance of the lowest possible pressure potential, and hence, the thinnest possible moisture films at the front consistent with continuous moisture films behind the front.

5. We may adapt the explanation given by Adam (*1*) for the rise of water in capillary tubes to the rise of water by capillarity through a porous medium such as soil, although it is recognized that the capillary tube is filled with water back of the advancing meniscus, and in unsaturated soil the water films are only partly bounded by a solid.

A. The liquid is not pulled through the soil by a hypothetical tension acting on the film which clings to and climbs up the surface of the soil particles.
B. The energy relations determine what the stable contact angle shall be.
C. The fluidity of the liquid permits the molecules to move about until they generate that angle.
D. The contact angle and the dimensions and shape of the voids between the solid particles, determined by the effective texture and packing arrangement, govern the curvature of the liquid-air interface.
E. The pressure difference arises from the free energy resident in the liquid-air interface.
F. The liquid then flows up under the hydrostatic pressure.

Disregarding for the present the translocation of water through soil in the vapor phase and the possibility of the establishment of continuous

moisture films through the agency of vapor flow, we may characterize the wetting front and some of its relations to the soil-moisture system in general from the five preceding observations:

1. The wetting front represents an irregular surface of discontinuity with continuous moisture films behind the front, and discontinuous or nonexistent films beyond the front.

2. At moisture contents below that characteristic for the wetting front, the capillary permeability of a soil to water in the liquid phase is zero. At these lower moisture contents the films are discontinuous, and there is no mechanism for liquid flow.

3. The magnitude of the pressure potential gradient from the dry soil to the wetting front has no influence on the rate of advance of this front except that a wetter soil requires less water to establish continuous films than a drier soil. The advance of the wetting front is by liquid flow, and is proportional to the pressure potential gradient back of the wetting front. The water flows under hydrostatic pressure.

4. At a given temperature, the curvature of the moisture films at the wetting front is characteristic for the soil solution and the soil, and does not vary with elevation of rise.

5. The moisture content at the wetting front is constant for a given soil solution and soil.

6. At constant temperature, the pressure potential at the wetting front is constant for a given soil solution and soil.

7. Water cannot rise by capillarity beyond the elevation at which the sum of the gravitational potential, the osmotic potential, and the pressure *potential characteristic of the wetting front* is equal to zero.

All the considerations thus far with reference to the wetting front have concerned only liquid flow through soils without the necessary intervention of vapor flow which is always present in a three-phase system. The soil has also been considered as a granular solid of constant effective texture and structure. The changes in the effective texture and structure with wetting cannot be completely described. It is known, however, that the mass effect of these changes is relatively insignificant in coarse-textured soil, but may be of considerable magnitude in other soils. Swelling of the colloidal fraction due to wetting is the only change in the solid phase that will be considered.

With the wetting front advancing rather rapidly through air-dry soil, the structure and effective texture immediately beyond the wetting front is probably very much the same as that which is characteristic for the

dry soil. When the wetting front approaches its maximum height above the water table and advances more slowly, wetting of the soil in advance of the front through the agency of vapor flow is of greater relative magnitude, the soil colloids swell, a lesser radius of curvature of the liquid surface may establish continuous moisture films, and the pressure potential of the wetting front may decrease progressively as the front advances from the water table to the maximum height of capillary rise. Experimental evidence is not at hand to permit a quantitative evaluation of the factors in the above speculation. For the present it seems worth while to bear these factors in mind, although they have not been determined, and though eventually they may be found to be of little significance.

Unsaturated permeability cannot be separated experimentally into its components of vapor and liquid permeability. If, however, we assume that vapor permeability for a given soil is constant when liquid permeability is zero, then liquid permeability would be zero when $\partial K / \partial \psi = 0$. In figure 19 the permeability of four soils is plotted as a function of the pressure potential. The permeability at each potential is expressed as a percentage of the permeability at zero pressure potential. The values for the pressure potentials at which capillary permeability becomes zero, as determined from moisture samples taken at the wetting front, are: Yolo fine sandy loam, — 92 gm–cm/gm; Yolo light clay, — 120 gm–cm/gm; and Yolo clay, — 140 gm–cm/gm. These potentials referred to the curves of $K = f(\psi)$, however, are not at values of ψ at which $\partial K / \partial \psi = 0$. But below these potentials both K and $\partial K / \partial \psi$ are very small. It is indicated that the potential at the wetting front represents, at least to the first approximation, the potential below which discontinuity occurs in the soil-moisture system, and is a critical point on the permeability curve at which capillary permeability becomes approximately zero. It is also indicated that the determination of the wetting front potential by sampling for moisture content is a practicable experimental procedure. This is worthy of further investigation.

INTERPRETATION OF SOME SOIL MOISTURE PHENOMENA IN TERMS OF PERMEABILITY

The Moisture Equivalent.—The moisture equivalent has been considered as representing a point on the pressure potential curve at which the pressure potential gradient of the soil is in approximate equilibrium with the centrifugal force applied, or at which the pressure potential is equal to —1000 gm–cm/gm (*21*). According to the data presented in this paper, the pressure potential at the moisture equivalent is much greater than — 1000 gm–cm/gm, varies with the texture of the soil, and repre-

sents the approximate ψ on the $K = f(\psi)$ curves at which the water in the soil becomes discontinuous, and the capillary permeability becomes zero.

Vapor permeability is high in sands. In centrifuging, the combined vapor and liquid flow reduces the moisture content of sand in the centrifuge cup to a pressure potential below that at which capillary flow ceases. In heavy clay the vapor permeability is low. The combined vapor and liquid flow is not sufficient to reduce the pressure potential of heavy clay to the point at which capillary flow ceases. The fact that the moisture equivalent of clay is generally higher, and of sands is generally lower than the field capacity would be expected from the above considerations of permeability.

Hysteresis of Curves of pF $= f(P_w)$.—An adaptation of a technique originally proposed by Bouyoucos (*5*) has been used for the derivation of data for the development of pF $= f(P_w)$ curves. The equipment required in this technique is: a source of vacuum, a Büchner flask, and filter paper coated with a thin layer of silt. The silt-coated paper forms a porous plate of small pore dimension. To determine the drying pF, a thin layer of soil is placed on the filter paper, and a continuous liquid phase is established extending through the filter plate, funnel, and stem, and into the filter flask. A constant vacuum is applied to the flask for a time considered sufficient to remove excess water. At the end of the drying time the soil is removed from the filter and the moisture content determined. The pF at that moisture content is assumed to be equal to the logarithm of the negative pressure applied. A drying curve of pF $= f(P_w)$ is developed from values of pF and P_w derived by repeating the procedure at different suction pressures. The technique for deriving the wetting pF is the reverse of that used in drying. In wetting, air-dry soil takes up water against a constant negative pressure maintained in the continuous liquid phase.

The assumptions necessary for the application of pF data derived by the above technique are:

1. At the end of the wetting or drying time, the pressure in the soil moisture films is at approximate equilibrium with the negative pressure applied.

2. The moisture films are continuous from the water in the flask through the soil layer.

The first assumption can only be valid within that moisture range in which the second assumption is true, since this technique is an approximate and rapid method for determining pF, and is applicable only if

water transfer can take place in the liquid phase. The time allowed for each determination is obviously too short to allow establishment of equilibrium moisture conditions through the agency of vapor flow.

The above-described technique, used at pressure potentials below which capillary permeability equals zero, would give erroneous results. On drying, the soil would lose water readily down to the pF at which the capillary permeability becomes zero. Further loss of water would be very slow through the agency of vapor flow. The experimental drying moisture content and pF would be higher than the true values representing equilibrium with the suction applied. On wetting, no capillary flow could take place, and the soil would receive water only by condensation of water vapor. The experimental wetting moisture content and pF would be lower than the true values representing equilibrium with the suction applied.

Curves of pF $= f(P_w)$ derived by the above technique show hysteresis of considerable magnitude between the wetting and drying curves. At a given pF the moisture content in the drying curve is much higher than that in the wetting curve. It is suggested that many of the data were secured in the moisture range in which the moisture films were discontinuous and the capillary permeability was zero; and that approximate pressure equilibrium was not established between the soil water and the water in the filter pores.

Moisture Distribution in Stratified Soils.—It is a well-known fact that a heavier-textured soil, underlaid by a coarse-textured soil, has a higher field capacity than the unstratified heavier soil (*2*). This phenomenon can be explained on the basis of the characteristic pressure potential for each soil at which capillary permeability becomes zero. To illustrate this point, let us consider two columns of soil as follows:

1. Column of unstratified Yolo clay
   A. K is approximately zero at $\psi = -140$
   B. $P_w = 26$ per cent at $\psi = -140$.

2. Column of Yolo clay stratified with Oakley sand; K of Oakley sand is approximately zero at $\psi = -80$.

After irrigation of the unstratified Yolo clay, water will flow downward under the influence of gravity until the moisture films become discontinuous. The final moisture distribution in the soil, neglecting vapor flow and in the absence of a water table, would be at $\psi = -140$ and $P_w = 26$ per cent.

After irrigation of the stratified column, water will flow downward through the clay with a pressure potential at the wetting front of ap-

proximately — 140. When the wetting front reaches the sand, flow cannot take place until the potential in the clay becomes greater than — 80 which is the minimum pressure potential at which flow is possible in the sand. (For Yolo clay $P_w = 30$ at $\psi = -80$.) The equilibrium moisture distribution in the clay, neglecting vapor flow, will be:

Distance above sand in centimeters	ψ	P_w
0	—80	30
0 to 60	-80 to — 140	30 to 26
Above 60	—140	26

If we assume the same two soil columns, ideally, under conditions of capillary rise:

1. With the top of the sand stratum less than 80 centimeters above the water table, the height of capillary rise will not be affected. At equilibrium, capillary water will rise to 140 centimeters above the water table.
2. If the bottom of the sand stratum is at any elevation between 80 to 140 centimeters above the water table, capillary rise will stop at 80 centimeters or at the bottom of the sand stratum.
3. If the sand stratum occurs so that a point 80 centimeters above the water is in sand, then capillary rise will reach its maximum in the sand at 80 centimeters above the water table. Theoretically, a shift of a few millimeters in the elevation of the sand stratum may make a difference of 60 centimeters in the maximum height of capillary rise.

SUMMARY

Data on the permeability of soils to water under saturated conditions is abundant; however, the unsaturated permeability of soils has received relatively little study, and published data on the subject are meager. The introduction of the potential function gave rise to the dynamic method in the study of unsaturated flow, and the development of the tensiometer instrument has made possible the direct determination of pressure potentials.

All published data now available on the permeability of soil to water in unsaturated flow were derived by the dynamic method from pressure potentials determined directly from tensiometers placed in the soil. This method was used in the soil-permeability studies reported in this paper.

Six soils, ranging in texture from sand to clay, were investigated under laboratory conditions of capillary rise. The soils were placed in metal cylinders with water supplied to their bases at a constant pressure sufficient to establish saturated conditions in the lower portion of the columns. Water flowed upward through the columns under the influence

of positive hydrostatic pressures in the saturated zone, and of negative hydrostatic pressures, or by capillarity, from the water table to the surface of the columns where it was removed by evaporation.

Pressure potentials in the unsaturated soil were studied as functions of moisture contents by determining the pressure potentials, ψ in gram-centimeters per gram, directly from tensiometer readings and the moisture contents, P_w, by sampling the soil columns. The relation between ψ and P_w for each soil was represented graphically in curves of $\psi = f(P_w)$. Hysteresis in the relation of ψ to P_w was found for all the soils, according to whether they were wetting or drying. At a given P_w, ψ was less after drainage than during the wetting process. The data of this experiment also indicate that for soils drying, the pressure potential depends upon the range through which the soil has dried.

The pressure potential in an unsaturated soil at constant moisture content increases with increasing temperature, and the amount of water held in the soil at a given pressure potential decreases with increasing temperature. During periods of temperature change in a soil column in which there is a high water table, these relations cause wide variations in the rate of water uptake from a water table. Under field conditions, rapid changes in soil temperature above a water table would be accompanied by rising water tables with rising temperature, and falling water tables with falling temperature.

The pressure potential of the water films in a soil is a measure of the curvature of the films and an index of the degree to which the soil is saturated. The relation of soil permeability, K, to pressure potential was studied and curves of $K = f(\psi)$ were developed. K could also be studied as a function of P_w through the relationship between ψ and P_w. Permeability was a maximum at or near saturation and decreased rapidly with decreasing P_w to approximately the moisture equivalent of the soil, at which moisture content the permeability was very low and remained constant or decreased only slightly with further decreases in moisture content. At this point $\partial K / \partial \psi = 0$ (approximately). This moisture content is also approximately that of the wetted front generated as the water advanced upward through the dry soil above a water table. These two criteria, the P_w at which $\partial K / \partial \psi = 0$ and the P_w of the wetting front, are interpreted as representing the P_w at which the moisture films in the soil become discontinuous and at which the capillary permeability of the soil is zero.

The texture of a soil affects permeability by its influence on the size, number, and continuity of the interspaces or pores. For soils of a like nature, such as the members of a single soil series, the size of pores de-

creases and the number of pores increases with increasing fineness of texture. The soils used in this experiment, arranged in order of permeability are, for saturated flow

sand > fine sandy loam > light clay > clay

and for unsaturated flow at $\psi = -100$

sand < fine sandy loam < light clay < clay.

If arranged in order of decreasing pressure potential at which unsaturated flow is approximately zero, the sequence obtained is:

sand > fine sandy loam > light clay > clay.

Knowledge of the variation of permeability with texture, especially the pressure potential at which capillary permeability is approximately zero, is fundamental to the consideration of the relative rates and maximum height of capillary rise, to the field capacity in stratified and non-stratified soils, and to single-valued "constants" such as the moisture equivalent, the field capacity, and the normal moisture-holding capacity. These experiments indicate that the pressure potential of the soil moisture at these constants varies with the texture of the soil. They are at the approximate ψ on the curves of $K = f(\psi)$ at which the moisture films in the soil become discontinuous and the capillary permeability becomes zero.

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SOIL MACROSTRUCTURE AS AFFECTED BY CULTURAL TREATMENTS

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INTRODUCTION

THIS INVESTIGATION was undertaken to find a quantitative method of expressing the structural condition of the soil, and by means of this method to study the effects of various mechanical treatments with respect to changes in structure. The effects of irrigation and of tillage operations were of particular concern. Seasonal changes were also observed.

Shaw (*17*)[3] has defined soil structure as:

> A term expressing the arrangement of individual grains and aggregates that make up the soil mass. The structure may refer to the natural arrangement of the soil when in place and undisturbed or to the soil at any degree of disturbance. The terms used indicate the character of the arrangement, the size and shape of the aggregates, and in some cases may indicate the consistence of those aggregates.

As thus defined, the term "soil structure" is obviously descriptive, and as such is not capable of being expressed by any specific measurement or number.

Many measurements of the physical properties of soils, which are dependent on the structure, have been made; and any of these measurements may be considered as an index of soil structure. These measurements are made either of the macro- or microstructure. A greater amount of work has been done on the microanalysis, which has been mainly measurements of the size distribution of particles after the soil has been slaked in water. The macroanalyses have been mainly measurements of the size distribution of coarse aggregates of soils obtained under field conditions in an undisturbed state. This present investigation is of the latter type; the method will be described in detail later. Other methods, such as the pull on the drawbar of a tillage implement, the amount of pressure required to force a sharpened instrument into the soil, and porosity measurements such as water penetration, air movement, so-called "capillary and noncapillary" pore space, and measurements of volume weight, have been used as indexes of soil structure.

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[3] Italic figures in parentheses refer to "Literature Cited," at the end of this paper.

The work here reported was carried out on soils of the Yolo, Denverton, and Capay series, on the University Farm at Davis, California, and on private farms nearby. Quantitative measurements were made of the changes in the size distribution of aggregates after tillage operations and irrigations, and of changes directly attributable to changes in the seasons. The studies on the effect of tillage operations were made in orchards, in fields in the preparation of seedbeds, and in test plots for special-study purposes.

The tillage operations in orchards were for the express purpose of controlling weeds. Those in the preparation of seedbeds were variable and dependent upon the season in which the crop was to be planted and the nature of the crop itself. In some of the seedbed preparations, preirrigations were made to insure an adequate moisture supply before seeding.

REVIEW OF LITERATURE

In this brief review of literature, no attempt is made to discuss all of the work that has been done on soil structure. A few papers dealing with each phase are mentioned, even though some of them bear rather indirectly on this investigation.

Russell (*15*), at Rothamsted, investigating the factors of importance in crumb formation in soils, found that clay particles can form strong aggregates or crumbs when dry, only if the clay particles are sufficiently small, if there are a sufficient number of small exchangeable ions on the clay, and if the clay has been dried from a dispersion medium whose molecules are polar and sufficiently small. Tiulin (*18*) in Russia, Demolon and Henin (*9*) in France, Novak (*14*) in Czechoslovakia, Bouyoucos (*2*), Baver and Rhodes (*1*), and Cole and Edlefsen (*6, 10*) in the United States, all have measured water-stable aggregates by sedimentation, or by wet sieving and sedimentation, as indexes of soil structure. In most cases, these workers recognize that certain aggregates are fairly stable in water, and that in some cases very drastic treatments are necessary to break them down materially. Demolon and Henin (*9*) and Tiulin (*18*) describe soil aggregates as being of two kinds: those that are water-stable are formed with clays whose base-exchange cations are dibasic, and those stable only in the dry condition are formed from compression, or with clays whose base-exchange cations are monobasic. All of the workers referred to are in accord in the belief that the formation of aggregates takes place as soils are being dried out. Vilensky (*20*) has shown that soils which give the greatest stability against slaking and require the greatest force for deformation of dry aggregates have definite moisture ranges.

In macroaggregate analysis, the work of Keen (*12*) and his associates is among the first. In this work, samples were obtained during tillage operations by cutting out rough cubes of soil with a spade and transferring them to a nest of sieves. The sieves were gently shaken a definite number of times and the material retained on each sieve was weighed. Chapman (*5*) observed that two plots on the North Dakota Experimental Farm were widely different in structure and measured this difference by sieve analysis of air-dry samples taken from each plot. Hoffman[4] determined the size distribution of aggregates before and after tillage with various kinds of implements. He used a 14-inch cylinder, which was driven into the soil to the depth of tillage and then inserted a steel plate under the cylinder to permit the removal of the soil in an undisturbed condition. The sample was then transferred to a graded nest of sieves that were shaken by hand and the weights determined. He was able to get good agreement between replications with the few tests made.

Keen (*12*) and his co-workers have used the drawbar pull on tillage implements which they measured with dynamometers to indicate the differences in soil structure. They also used the depth of penetration of a sharpened instrument into the soil under definite impact as a means of measuring the compaction. Davis (*8*) has further perfected this apparatus with a mechanical data recorder, and has used it extensively. Another even simpler and less expensive implement of this type has been designed and used by Culpin (*7*). This instrument also has a self-recording attachment.

The measurement of the porosity of soil by permeability of gases and liquids has been used as a means of studying certain physical properties. Bouyoucos (*3*) has devised a method of measuring water penetration by slaking a definite quantity of soil on a filter paper in a Buchner funnel and then applying suction. Methods of studying permeability by means of various kinds of tubes are too numerous to mention here. Buehrer (*4*) at Arizona has devised a method of passing air through soils under definite conditions of compaction, and using the values thus obtained to define soil structure. Dojarenko (according to Krause, *13*) in Russia has measured the so-called "capillary and noncapillary" pore space as an expression of the soil structure, considering these values to be much more expressive of the physical properties of field soils than volume-weight expressions where the total pore space alone is calculated. The determination of capillary and noncapillary pore space is made by taking a column of soil 10 cm high, setting it on a piece of filter paper that is in contact with a free water surface, and permitting the soil to absorb as

[4] Hoffman, A. H. Unpublished manuscript.

much water as possible in 48 hours. The volume of water retained under these conditions is known as "capillary pore space" and the remainder of the pore space is considered "noncapillary pore space." He designed a special tube 10 cm high with a volume of 100 cc for obtaining samples in an undisturbed state. The principal objection to this measurement is that the amount of water that can be absorbed by any given soil will depend on the height of the column.

EXPERIMENTAL METHODS

Soils.—The soils on which experiments were carried out are of the Yolo, Capay, and Denverton series. These are all mineral secondary soils derived from sedimentary rock sources.

The Yolo soils are recent alluvial soils occupying smooth, gently sloping alluvial fans. There are wide variations in textural types within this series. Normally the surface soils are friable, but they become easily puddled under improper management. The subsoils are loose and friable, and of various textures. Stratification is common in the subsoil. Drainage is adequate and root and water penetration through the soil is excellent.

The soils of the Capay series are closely related to those of the Yolo series but are found further out on the alluvial fans with flatter relief and somewhat restricted drainage. The surface textures are heavy, usually of clay texture, and exhibit an adobe structure. When wet, these soils are very sticky, and when dry, large cracks occur which leave the soil in large hard adobelike blocks. The subsoil is of somewhat heavier texture than the surface soil, and is considerably compacted. This greatly inhibits root and water penetration. During the rainy season, water often stands in pools on the surface for a considerable period of time. As the water disappears from the surface, the surface soil dries rapidly and adobe cracks form, but the subsoil remains saturated for a considerable period of time after the surface soil becomes dry. The water table is often found within 6 feet of the surface in these soils.

The Denverton soils occupy high rolling terraces, often with fairly steep slopes. The profiles are immature to semimature in stage of development. Surface textures are usually heavy clay loams or clays that have definite adobe structures. Unlike those of the Capay soils, however, the adobe blocks have a large number of secondary cracks. This condition renders the surface soil very friable. The large adobe blocks break down readily to small angular units which are fairly stable. The subsoil is somewhat compact yet it is moderately permeable to roots and water. The physical properties of these soils are far more favorable to root and

water penetration and to cultural treatments than the soils of the Capay series. Owing to their irregular relief, however, they are seldom brought under irrigation.

Method of Sampling.—The samples were taken to tillage depth in the case of cultivation tests, and to specified depths in the case of irrigation tests and studies of seasonal variations, by driving a 14-inch steel cylinder into the soil, excavating the soil from around the cylinder, and driving a piece of sheet steel under it to remove the samples without disturbing the structure. The samples thus obtained were carefully transferred to orchard lug boxes and permitted to dry slowly under shelter.

In 1935, the percentages of moisture and the volume weights were obtained at the time the samples were collected.

Volume-Weight Determinations.—During the 1935 season, a method was found for determining the volume weight of the soil as samples were being taken for aggregate analyses. The volume weight was measured by using the same cylinder and sample as was used for sampling for aggregate analysis. After a sample had been removed from the ground as described under "Methods of Sampling," a straight-edge was placed across the top of the tube, and from 75 to 100 measurements, spaced systematically over the surface, were made from this straight edge to the surface of the soil in the cylinder. This permitted calculating the portion of the cylinder unoccupied by the sample. The difference between this figure and the total volume gave the volume of the sample. Aliquot moisture samples were taken in duplicate, from the soil right next to where the sample had been removed, and these were used as the moisture content of the sample in calculating its volume weight. The entire sample was weighed as it was taken from the field, and from the data thus obtained the volume weight was calculated. Replicates agreed within 5 per cent, which is considered good for this type of sampling. In all volume-weight determinations here reported the figures quoted are averages of 4 replicates.

Method of Sifting.—The air-dry samples were sifted in two stages: first through a nest of very coarse sieves, then in a Ro-tap shaker.

The nest of coarse sieves (designed especially for this purpose) is made of wooden boxes 8 inches deep and 2 × 3 feet inside dimensions with rods or pipes to form the sieves, the diameters being proportioned to the size of the opening. The coarsest screen has 1⅛-inch rods set 6 inches apart on center, which gives openings of 4⅞ inches; the next has ¾-inch rods 4 inches apart on center, which gives openings of 3¼ inches; and the third screen has ⅜-inch rods 2 inches apart on center, which gives 1⅝-inch openings. The fourth is a box of the same size with a solid bottom,

in which the material passing through the finest sieve is caught. These are nested in a frame and held in place by dowels. This frame is mounted on wheels which fit on tracks of 2-inch angle iron. The tracks have stops at each end that permit a 24-inch movement of the nest of sieves. These tracks are mounted on rockers, which rock the frame while it is being rolled along the tracks. Twelve shakings of this sieve are sufficient to separate the sample to its various sizes.

The material collected in the solid box of the large shaker was sifted in a Tyler Ro-tap shaker. The sieves have openings of approximately $\frac{3}{4}$, $\frac{3}{8}$, $\frac{3}{16}$, $\frac{3}{32}$, $\frac{3}{64}$, and $\frac{3}{128}$ inch. The first two are unnumbered, but the last four are 4, 8, 14, and 28 mesh, respectively. The material fine enough to pass through the finest mesh was caught on a solid pan that could be nested with the sieves. As only 3 pounds of soil can be shaken in this machine at one time, a number of determinations were necessary for each sample.

The Ro-tap shaker is operated with an electric motor and has a speed of about 150 shakes a minute. There is a horizontal movement of about 2 inches, and at the same time a vertical displacement of about 1 inch caused by a hammer that bounces the sieves up and down. Fifty shakes were found to be sufficient to sift a sample (see p. 437).

The soil aggregates have irregular shapes, yet they are compared as if they had regular shapes. Since their exact dimensions are not known, the calculation of the surface cannot be made in definite units, but only in relative terms, and so the comparison of surfaces on all the fractions of the sample is called "relative surface" and is expressed in nondimensional units.

In using the relative-surface values as here calculated, two assumptions are made: (1) that aggregates within the same sample of soil have the same volume weight; (2) that the aggregates are cubes.

The value is calculated on the actual size of the sieve opening, which is the minimum size of particles in any size range, except for the fraction which passes through the finest sieve. Here an arbitrary value of one-half that of the finest sieve is used. These minimum values are proportional to the mean values for the size ranges and hence will not affect the ratio for the various sieve openings.

Since the same set of sieves was used for all of the sieve analyses, the value is a constant for any one fraction. The values for these fractions are as follows: through $\frac{3}{128}$ inch, 416.0; $\frac{3}{128}$ inch, 208.0; $\frac{3}{64}$ inch, 104; $\frac{3}{32}$ inch, 52; $\frac{3}{16}$ inch, 26; $\frac{3}{8}$ inch, 13.0; $\frac{3}{4}$ inch, 6.5; $1\frac{5}{8}$ inches, 3.0; $3\frac{1}{4}$ inches, 1.56; and $4\frac{7}{8}$ inches, 1.0.

The relative-surface values are obtained according to the following formula:

$$RS = \sum \left(\frac{O_1}{O_1} M_1 + \frac{O_1}{O_2} M_2 + \ldots . \frac{O_1}{O_n} M_n \right),$$

where:

RS = relative surface
O_1 = sieve opening of the coarsest sieve
O_2 = sieve opening of the next coarsest sieve
M_1 = per cent of sample retained on the coarsest sieve
M_2 = per cent of sample retained on the next coarsest sieve.

The finest fractions have such a great influence on the values for relative surface that small differences in the percentage values for these finer fractions greatly affect this value, whereas large differences in the coarser fractions do not alter it very much.

Two soils with entirely different size distributions of aggregates may have very close relative-surface values; and, likewise, other soils that have very similar size distribution of particles may have relative-surface values that are not so close. If the percentage values for the three finest fractions are very much alike, the relative-surface values will not be greatly altered by a drastic change in the size distribution of the other seven fractions. In order to get a more complete picture of the changes that occur within any soil, both the figures for the size distribution of aggregates and the values for the relative surface are presented in form of tables, or, where the data on size distribution of aggregates are plotted as curves, the values for the relative surface are given in tabular form with the legend for the curves.

Plotting of Data on Graphs.—Many of the results here presented are plotted on graphs, which afford an easy way of making comparisons. The sieve openings are plotted as abscissas and summation percentages as ordinates. Summation percentages are used instead of the percentages on each sieve because the curves thus plotted are much easier to compare. When curves on the same graph are compared, the curve highest on the graph has the finest aggregates.

In the lower right-hand corner of each graph, the data for the four finest fractions are replotted on a larger scale: the scale for the abscissa is 10 times, and that for the ordinate 2 times, the respective scales on the main graph.

EVALUATION OF THE METHODS USED

Effect of Sifting.—As the term is used in this discussion, a "soil aggregate" is composed of a number of individual soil particles that form a mass sufficiently stable to act as an individual unit.

Many of the clods had rather large cracks as the sample was placed on the large sieve. In most cases, the sifting manipulation was severe enough to break the clods apart at the cracks into aggregate units, but not severe enough to break up uncracked units. Careful observations of the clods on each sieve revealed that very few of them showed any cracks, yet their surfaces did not show fresh breaks, which was taken to indicate that very few aggregate units were broken in this process.

The amount of shaking will obviously affect the results: if inadequate, the sample will not be completely separated; if too prolonged, the aggregates will be broken or worn down.

The data recorded in table 1 show two tests on Sacramento adobe clay with the Ro-tap shaker. The samples were shaken 50 times in the usual manner and the weight on each sieve recorded. The material was reassembled and the process repeated, this time with 200 shakings. The data were again recorded and the process repeated several times, with 250 shakings at each repetition thereafter, until 1,500 in all had been given the samples.

The percentage on the intermediate and finer sieves ($3/16$, $3/32$, $3/64$, and $3/128$ inch) did not change very much. The two coarsest sieves lost some material, and the solid pan gained a little by each successive series of shakings. These results indicate that although there is some wearing down of aggregates by shaking, it is more a case of grinding off corners than breaking down aggregates. A careful examination of the aggregates at the end of this prolonged shaking showed that, especially on the three coarsest sieves, they are well rounded, having almost the appearance of water-worn gravel. These data clearly show that the aggregates are sufficiently stable to be measured in this manner, and that 50 shakes of the Ro-tap are enough to separate the sample properly into the various-sized units.

Variability of Samples.—A certain amount of variability in the samples is to be expected, so that no one determination will convey a true picture of the state of aggregation of any soil. An average of several determinations will more accurately describe this condition, and all of the data presented are averages of four or more replicate samples.

Table 2 shows differences between 8 individual samples on each of three different sets taken in an orchard on Yolo loam as the field was

being cultivated. The first set was taken before any tillage operation, the second after the same strip had been cultivated in one direction with a heavy disk harrow to a depth of 4 inches, and the third after it had been cross-cultivated with the same implement at right angles to the first operation. The cultivations were made on the same day, within a few hours

TABLE 1

PERCENTAGE DISTRIBUTION OF SOIL AGGREGATES AFTER VARIOUS NUMBERS OF SHAKINGS IN THE RO-TAP SHAKER

Number of shakings	Sieve opening							Total
	Through 3/128 inch	3/128 inch	3/64 inch	3/32 inch	3/16 inch	3/8 inch	3/4 inch	
	First test							
50	15 1	13 0	18 1	17.5	16 5	16 7	2 9	99.8
250	16 8	13 5	18 7	17 3	16 5	14 0	2 7	99.5
500 ..	17 5	13 9	19.2	17.5	16.0	13 7	1 8	99.6
750	18 1	14 0	19.2	17 9	16.0	12 5	1 8	99.5
1,000	18 6	14 1	19 6	17.9	15 8	11 7	1.8	99.5
1,250	18 9	14 3	19 6	18.0	15 7	11 2	1 8	99 5
1,500	19 4	14 3	19 7	18 0	15 5	12 0	0 8	99.7
	Second test							
50	15 8	12 3	16.9	16 0	16 4	19 6	2 9	99 9
250	17.5	12 8	17 1	16 3	15 8	17.6	1.9	100.0
500	18.0	12 8	17.4	16 5	15 5	17 4	1 9	99.5
750 ..	18 7	12 8	17 4	16 5	15 8	16 7	1 7	99.5
1,000	19 2	13 1	17 4	16 6	15 3	16.2	1 0	98.8
1,250	19 5	13 2	17.4	16 8	15 6	15 1	1 0	98.6
1,500	20 1	13 1	17 6	16 9	15 5	14 4	1 0	98.6

of each other, and all of the samples were taken within an area about 20 feet square.

Definite pulverizing effect is obtained by each tillage operation, yet there is considerable variation between individual samples for each treatment. There is usually a greater variability in the coarser fractions than in the intermediate and finer fractions. Some variability may be ascribed to the method of making the determinations, but much more of it is due to the natural heterogeneity of the soil, so that an average of a number of replicate samples for the same treatment is the only safe way of making comparisons between different cultural treatments.

Comparison of Moist and Air-dry Sifting.—Keen (*12*) and Hoffman[5] sifted their samples as they were taken from the field. In the present study, because comparisons were to be made between samples taken from

[5] Hoffman, A. H. Unpublished manuscript.

fields under variable conditions of texture and moisture, and also at different seasons, it was desirable to bring the samples to moisture conditions as nearly comparable as possible. The air-dry state is most con-

TABLE 2

Variability in Percentage Distribution of Soil Aggregates in Individual Samples of Yolo Loam

Sample No.	Sieve opening										Relative surface
	Through 3/128 inch	3/128 inch	3/64 inch	3/32 inch	3/16 inch	3/8 inch	3/4 inch	$1\frac{5}{8}$ inches	$3\frac{1}{4}$ inches	$4\frac{7}{8}$ inches	
					No cultivation						
1	5.0	1.8	2.5	3.7	5.5	9.1	14.2	24.1	24.8	9.5	3,380
2	3.9	1.4	2.2	3.3	5.1	8.2	12.5	18.4	10.6	34.3	2,741
3	4.8	1.7	2.5	3.9	5.3	8.0	12.0	17.4	27.5	16.5	3,243
4	4.2	1.3	2.0	3.4	6.0	10.9	18.4	20.2	21.2	12.4	2,935
5	5.8	1.7	2.4	3.7	5.6	9.6	11.4	31.7	28.2	0.0	3,694
6	7.4	1.9	2.8	4.3	6.5	10.6	14.6	30.7	12.1	8.9	4,513
7	6.6	1.9	2.9	4.5	6.8	10.3	13.1	28.1	17.8	8.2	4,191
8	4.5	1.4	2.0	3.2	4.9	7.4	11.9	16.5	19.0	29.3	2,947
Av.	5.3	1.6	2.4	3.8	5.7	9.3	13.5	23.4	20.2	14.9	3,460
					Cultivated one way						
9	10.9	3.3	4.7	6.8	9.2	13.0	15.8	20.7	15.5	0.0	6,656
10	9.6	3.2	4.7	7.5	10.3	15.9	22.6	26.4	0.0	0.0	6,242
11	14.3	4.1	5.1	8.0	14.3	18.2	20.2	11.6	4.2	0.0	8,570
12	12.1	3.7	5.3	7.4	11.1	15.5	18.2	16.1	10.3	0.0	7,390
13	12.6	3.0	4.3	6.7	9.9	13.7	17.8	27.9	4.2	0.0	7,290
14	12.7	3.4	4.3	6.0	8.7	12.1	15.9	14.0	22.8	0.0	7,300
15	10.2	3.2	5.1	8.2	11.4	15.2	19.3	18.5	9.0	0.0	6,551
16	11.3	2.9	4.4	7.0	11.2	15.7	26.1	22.2	0.0	0.0	6,857
Av.	11.7	3.4	4.7	7.2	10.8	14.9	19.5	19.7	8.3	0.0	7,113
					Cultivated 2 ways						
17	17.7	5.6	8.1	11.7	14.7	17.9	14.6	9.8	0.0	0.0	10,724
18	12.7	4.6	6.7	10.0	13.6	17.8	22.4	13.2	0.0	0.0	8,285
19	16.9	5.4	7.2	10.1	13.6	17.4	17.8	11.6	0.0	0.0	10,158
20	14.2	4.8	6.8	9.0	13.0	17.1	25.4	9.5	0.0	0.0	8,829
21	23.1	5.8	7.3	9.6	11.7	13.5	13.7	12.9	2.2	0.0	12,678
22	15.3	5.7	8.3	12.5	17.9	20.9	15.4	3.7	0.0	0.0	9,986
23	14.5	5.9	8.2	11.0	13.6	16.0	15.1	12.2	2.5	0.0	9,393
24	18.6	7.0	8.9	11.3	13.5	16.2	15.8	4.7	4.1	0.0	11,393
Av.	16.6	5.6	7.7	10.7	14.0	17.1	17.5	9.7	1.1	0.0	10,203

venient, even though this necessitates storing the samples while drying.

A few tests were made to compare samples sifted at field moisture content and under air-dry conditions. The data (table 3) for samples from fields of Yolo loam and of Yolo silt loam are presented to show the

differences in samples taken at the same time, one set sifted immediately after sampling, and the other air-dried before sifting. Replicate samples agreed well when sifted moist as well as when sifted in the air-dry state. There were marked differences in the size distribution under these two systems of handling the samples. The amounts both of very coarse material and of fine material were always greater when sifted in the air-dry condition.

At field moisture content, the larger aggregates seem to have been weak enough to be broken by the amount of agitation necessary for complete separation with this apparatus. On the other hand, there was less material fine enough to pass through the finest sieve. The moisture films undoubtedly held the dust particles together in aggregates sufficiently stable to prevent their passing through the finest sieve on the Ro-tap shaker. In two respects, therefore, size distribution of aggregates as found by sifting the samples at their field moisture content is obviously different from that of the samples when taken from the field.

TABLE 3

PERCENTAGE DISTRIBUTION OF SIZE AGGREGATES SIFTED AIR-DRY AND AT FIELD MOISTURE CONTENT

Soil and plot No	Per cent of water	Sieve opening										Relative surface
		Through 3/128 inch	3/128 inch	3/64 inch	3/32 inch	3/16 inch	3/8 inch	3/4 inch	1⅝ inches	3¼ inches	4⅞ inches	
Yolo loam												
Plot 1	3.42	5.4	3.5	4.6	6.2	8.5	12.2	15.0	23.0	17.6	4.3	4,362
	20.9	1.6	7.3	12.2	11.4	13.9	17.3	18.1	13.8	4.9	0.0	4,791
Plot 2	3.06	6.2	3.7	4.8	6.2	8.3	11.3	14.3	23.5	21.6	0.0	4,718
	20.1	2.0	7.3	11.6	10.7	13.0	16.4	17.2	19.8	2.0	0.0	4,805
Plot 3	3.30	3.5	2.0	3.0	4.1	6.0	9.7	15.6	25.1	26.2	4.7	2,936
	21.5	0.3	3.3	9.5	9.4	11.5	15.9	18.3	25.5	6.3	0.0	2,993
Yolo silt loam												
Plot 1	3.10	6.5	2.5	3.0	4.3	6.5	10.1	14.1	27.3	19.9	6.2	4,259
	21.9	4.2	6.6	8.7	9.0	11.7	15.8	19.9	20.5	3.3	0.0	5,202
Plot 2	3.25	6.8	2.6	3.2	4.4	6.3	9.6	14.6	25.9	14.4	12.3	4,436
	21.7	3.4	8.5	11.3	11.0	13.4	17.6	19.3	14.1	1.4	0.0	4,632
Plot 3	3.18	3.8	2.3	3.1	4.4	6.6	10.5	15.4	24.3	23.7	6.0	3,124
	21.7	0.8	5.3	7.5	8.4	11.2	14.4	15.8	26.9	9.7	0.0	3,350

With the air-dry samples, on the contrary, the stability previously demonstrated (p. 437) for the air-dry aggregates indicates that the size distribution as found by sifting was essentially the same as that before sifting. It may be argued that there is a possibility for change in the size

of the aggregates as the samples dry out, but this phase was not investigated.

The relative-surface values under the two different sets of conditions are not greatly different.

For comparison of the degree of wetness of the various samples, the ratio of the moisture content to the moisture equivalent at the time of sifting is given in table 4. These soils were all sampled the same length of time after a series of irrigations, and were very close in their degree of wetness and actual moisture content.

TABLE 4

Ratio of Percentage of Water at Field Capacity to Moisture Equivalent in Yolo Loam and Yolo Silt Loam

Soil and location	Per cent moisture	Moisture equivalent	$\frac{\text{Per cent } H_2O}{\text{M.E.}} \times 100$
Yolo loam			
Plot 1	20 9	25 15	0 83
Plot 2	20.1	25.30	0.79
Plot 3	21.5	25.88	0 83
Yolo silt loam			
Plot 1	21.9	25.95	0 84
Plot 2	21.7	26.42	0.82
Plot 3	21.7	26.80	0.81

EFFECT OF TILLAGE ON MACROSTRUCTURE

Orchards.—Permanent crops, such as orchards and vineyards, are usually subjected to tillage operations for the purposes of turning under covercrops or destroying weeds. Usually this is done with disks of some type, either single or double, and the depth of penetration of the implement is rather shallow, 3 to 6 inches being the most common. In the present study, the implement was a heavy single-disk harrow, operated at depths of 4 to 5 inches. The second cultivation, when given, was at right angles to the first.

Table 5 gives the data for orchards on the Yolo series. In all cases, each tillage operation had a distinct pulverizing effect.

Yolo loam was sampled in three locations (table 5, plots 100, 200, and 300), and considerable variation was found in the size distribution at each location before any tillage operation was performed. In each case, there was a distinct pulverizing effect by each operation, but the intensity of this effect was variable.

The two locations on the Yolo clay loam (plots 400 and 500) were not

so variable before tillage, and the pulverizing effect of the tillage was also a little more uniform.

The soils with the coarser textures were less aggregated than those of the finer textures. Before tillage, Yolo fine sandy loam (plot 000) showed a much finer condition of aggregates than the other two soils. It had relative-surface values two or three times as great as the loam and five or six times as great as the clay loam.

TABLE 5

PERCENTAGE DISTRIBUTION OF SOIL AGGREGATES FROM DIFFERENT LOCATIONS AND SOIL TYPES IN AN ORCHARD

Plot No.	Cultivations	Sieve opening										Relative surface
		Through 3/128 inch	3/128 inch	3/64 inch	3/32 inch	3/16 inch	3/8 inch	3/4 inch	1⅝ inches	3¼ inches	4⅞ inches	
Yolo fine sandy loam: percentage distribution of aggregates												
000	0	21.8	3.8	4.6	6.6	8.9	10.1	9.0	12.5	5.0	17.7	11,157
	1	23.8	4.5	5.6	8.2	11.2	14.3	13.9	13.4	5.2	0.0	12,411
	2	29.7	5.9	7.1	9.7	12.2	13.5	11.6	10.6	0.0	0.0	15,420
Yolo loam: percentage distribution of aggregates												
100	0	7.2	2.0	2.6	3.7	5.4	7.3	9.5	17.8	12.7	31.8	4,273
	1	12.9	3.2	4.5	6.4	9.0	12.5	15.5	20.5	7.7	7.9	7,406
	2	14.7	4.0	5.5	7.6	10.3	13.6	14.7	17.7	4.9	6.9	8,506
200	0	5.8	1.7	2.1	3.3	4.5	6.7	9.8	16.1	12.7	37.2	3,506
	1	10.9	2.8	3.7	5.1	7.0	9.6	10.8	20.0	19.9	10.2	6,240
	2	23.6	7.6	8.6	10.0	12.7	13.8	12.4	9.9	1.6	0.0	13,435
300	0	12.2	2.9	3.5	4.5	7.1	10.4	13.3	17.5	19.2	9.3	6,778
	1	15.6	4.3	4.4	5.7	7.5	11.2	13.0	19.1	7.4	12.2	8,333
	2	19.5	4.7	5.3	7.1	9.4	14.0	16.2	14.6	9.5	0.0	10,597
Yolo clay loam: percentage distribution of aggregates												
400	0	2.3	1.3	1.9	2.7	4.1	6.2	8.4	12.5	22.5	38.1	1,916
	1	5.9	5.6	6.8	8.9	11.9	15.3	16.8	17.2	8.8	0.0	6,718
	2	13.0	7.1	8.7	10.7	13.5	15.9	14.8	13.1	3.2	0.0	9,025
500	0	2.6	1.6	2.2	3.2	4.6	6.8	8.0	10.9	19.4	40.6	2,175
	1	8.0	5.1	6.3	8.5	11.9	15.3	18.8	21.7	4.3	0.0	6,185
	2	10.4	6.5	7.4	9.0	11.5	14.5	15.1	19.2	6.5	0.0	7,563

Table 6 shows the results of two seasons' studies on tillage in an orchard on Denverton adobe clay. During the 1933 season, there was no noticeable pulverizing effect due to the tillage operations, whereas there was a slight pulverizing effect after the second cultivation in the 1934 season. The soil was in a more cloddy condition in 1934 than in 1935.

Despite its heavy texture, this soil showed a good granular structure. The soil cracks into large adobe blocks on drying out, but these large blocks have numerous secondary cracks that break the soil into rather small irregular-shaped clods that are themselves rather firm; hence, the tillage operation did not have a very strong pulverizing effect.

In order to compare more closely the size distribution of aggregates after the various treatments, the data from one set of samples from Yolo loam and one set from the Denverton adobe clay are plotted on graphs.

TABLE 6

PERCENTAGE DISTRIBUTION OF SIZE AGGREGATES BEFORE AND AFTER TILLAGE OPERATIONS IN AN ORCHARD ON DENVERTON ADOBE CLAY, 1933 AND 1934

Year	Cultivations	Sieve opening										Relative surface
		Through 3/128 inch	3/128 inch	3/64 inch	3/32 inch	3/16 inch	3/8 inch	3/4 inch	1⅝ inches	3¼ inches	3⅞ inches	
1933	0	13 9	13 3	15 2	14.6	14 4	12 3	7.7	7 3	1 4	0 0	11,510
	1	15 5	14.3	16.0	15.0	15 0	12 9	7 1	4 2	0.0	0 0	12,483
	2*	14 7	12 3	13.2	12 8	13.7	14.6	11 5	7 2	0 0	0 0	11,363
	After rain	13 8	12 6	14 0	13 6	14 6	14 3	11 6	5 6	0 0	0 0	11,159
1934	0	7 0	8 9	11.0	10 2	13 4	14 8	12.1	25.1	0 0	0 0	7,108
	1	7 3	9 2	12 1	12 4	14.1	16 3	28 5	0 0	0 0	0 0	7,622
	2*	10 7	11 8	15 4	13 9	14.1	15 6	18 5	0 0	0 0	0 0	9,895

* Second cultivation at right angles to first

Figure 1 gives the data for plot 300 on Yolo loam and shows the distinct pulverizing effect of each cultivation. The curves have similar shapes. The differences are also manifest in the relative-surface values.

Figure 2 shows the curves for the 1933 samplings on Denverton adobe clay. The size distribution of aggregates in these samples was not greatly altered by the cultural treatments. These curves occur much higher on the graph than those of the Yolo loam, which shows them to have finer aggregates. The curves are very close together and of similar shape. Curve *B*, after cultivation in one direction, is slightly higher than the other curves on this graph. This difference is also manifest in the relative-surface values. The other three curves cross each other a number of times and are very close together throughout the entire range of the curve. Their relative-surface values are also very close.

Seedbed for Beans.—In 1933 and 1934 a number of studies were carried out on a private farm in the vicinity of Davis, California. The studies were made on the size distribution of aggregates after different tillage operations in preparing seedbeds. This farm is composed mainly of soils of the Yolo series, ranging in texture from fine sandy loam to clay loam, but there is also a body of Capay adobe clay on this farm.

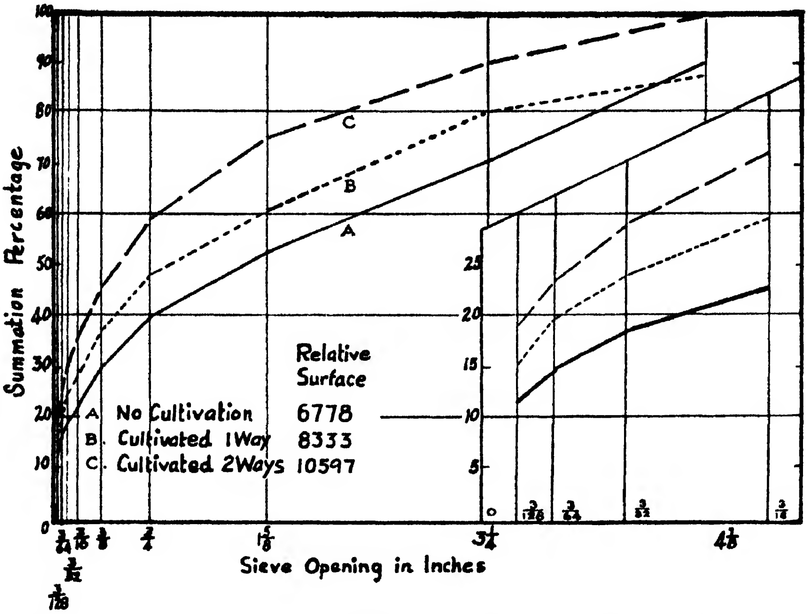

Fig. 1.—Size distribution of aggregates before and after tillage operations in an orchard (plot 300) on Yolo loam soil, 1933.

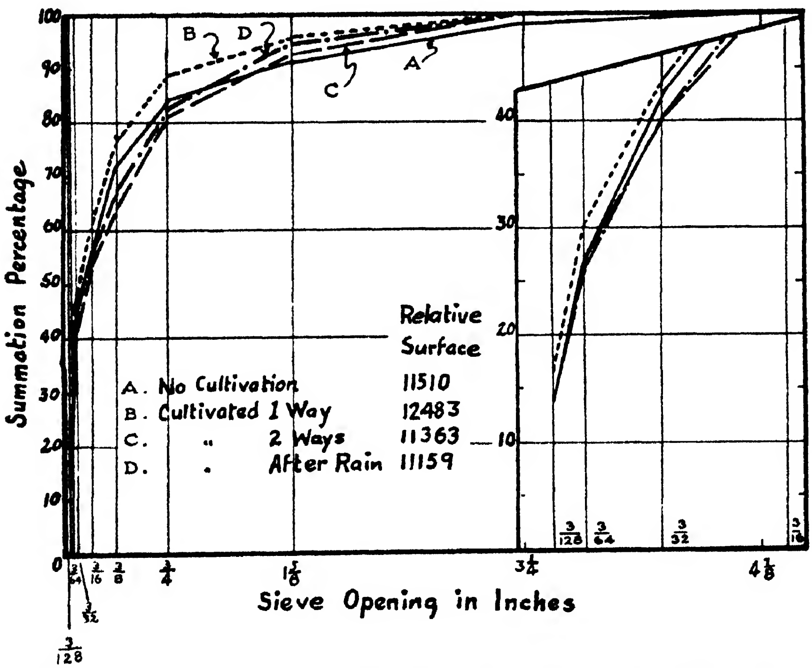

Fig. 2.—Size distribution of aggregates before and after tillage operations in an orchard on Denverton adobe clay, 1933.

The farm had been used for dry-land grain for a good many years. For four or five years previous to these investigations, the entire farm had been in sod and pastured to sheep.

In 1934 a field of considerable size was planted to beans. Two areas were selected for the investigations on the preparation of the seedbed: one area was on Yolo clay loam and the other on Capay adobe clay.

The bean field was worked as a unit, and the treatments on the whole field were the same. These were briefly as follows: The field was plowed in a fairly dry condition, to a depth of 6 inches. Some leveling was done next by use of a heavy float and a large scraper. After this, the field was preirrigated, permitted to dry at the surface for nearly a month, then harrowed with a spring-tooth harrow, and finally rolled with a cultipacker before seeding. The leveling work on this field did not necessitate the moving of very much soil except for small localized areas. No soil was moved to or from the areas in either sampling location.

All of the data for the studies of preparation of seedbed on this farm are plotted in graphs. Figure 3 shows the results of the treatments on Yolo clay loam and figure 4 shows those for Capay adobe clay. On the Yolo clay loam, the plowing had only a very slight pulverizing effect, but the same treatment had a very definite pulverizing effect on the Capay adobe clay. On both plots, the leveling operations had a very strong pulverizing effect, and the plots were in their most finely pulverized state after this treatment. After the preirrigation and subsequent drying period, both plots were far more cloddy than they had been before they had received any cultural treatment. On both plots, the harrowing with a spring-tooth harrow and rolling with a cultipacker had some pulverizing effect. The curves for the data on the Yolo clay loam (fig. 3) are of very similar shape. The three curves *A*, *B*, and *F* are close together, yet the corresponding relative-surface values are 4,940, 5,348, and 5,648, respectively. As previously noted, small changes in the percentage of the finer fractions cause large changes in these values. Curve *F* falls between curves *A* and *B* throughout most of its length, but the percentage of very fine fractions is higher in *F*, as shown on the enlargement at the lower right-hand corner of figure 3.

The similarity of curves *A* and *F* indicates that, for this soil, the size distribution of aggregates was practically no different when the field was seeded than it had been before plowing. The actual physical condition of the field, however, was greatly altered. The soil was dry and compact and in a fairly heavy sod before plowing. As the field was finally fitted before seeding, it was loose and friable and the sod had all been broken up. Unfortunately no volume-weight measurements were taken this season.

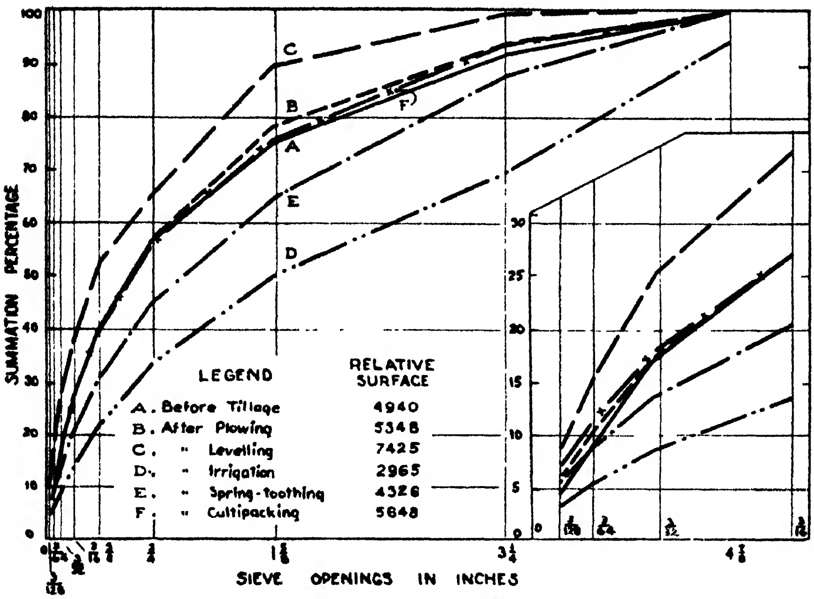

Fig. 3.—Size distribution of aggregates before and after tillage operations in preparation of seedbed for beans on Yolo clay loam, 1934.

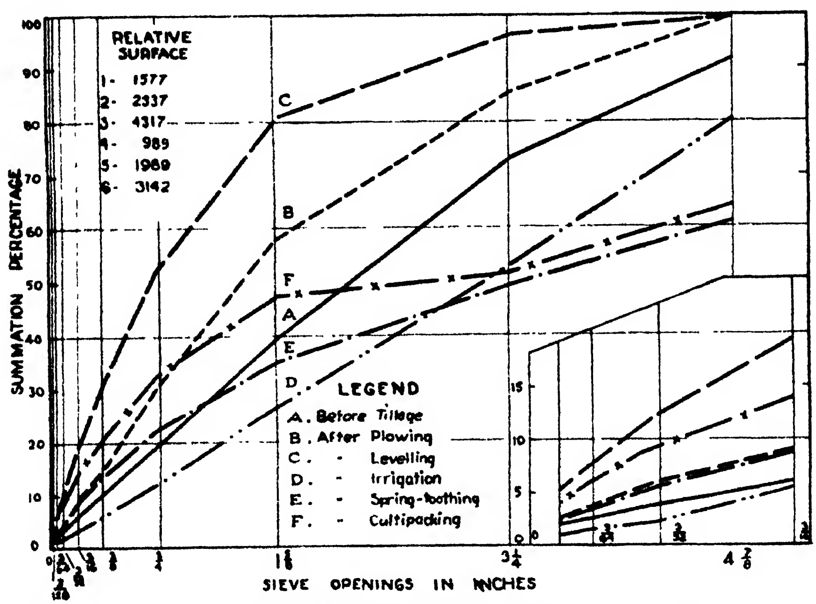

Fig. 4.—Size distribution of aggregates before and after tillage operations in preparation of seedbed for beans on Capay adobe clay, 1934.

The curves in figure 4 for Capay adobe clay are spread wider apart than those in figure 3. Curves *E* and *F* in this figure do not have the same general shape as the others. The field was permitted to dry for several weeks after irrigation. The Capay soils have very sluggish drainage, and water stood on this area several days longer than on the Yolo clay loam. As the field dried out after the water disappeared from the surface, a definite crust about 2 inches thick was formed; below this, the soil was very wet. The field was in this condition when the spring-tooth harrowing and cultipacking were done. In both instances, samples were taken to 4-inch depths. Harrowing increased the amount of fine material over that of the sample before any tillage was done, but also increased the amount of coarse material. The drier soil at the surface was somewhat crumbled, but the pressure of the implement caused the very wet soil under the crust to be pressed together, and this dried out into very coarse lumps. The subsequent rolling with the cultipacker had a rather strong pulverizing effect on the crust but did not affect the lower portion of this layer. Here again, the strong influence of the finer fractions is clearly shown. Curve *A* is above curve *E* for most of its length but somewhat below the latter at the beginning; yet it has a relative-surface value of 1,577 as compared to that of 1,989 for curve *E*. Curve *F* has a relative-surface value of 3,142, which is just double that for curve *A*; yet it lies above curve *A* for only about half its length and falls far below for the last half.

The size-distribution curves and relative-surface values show the Yolo clay loam to have much less cloddy structure than the Capay adobe clay.

Seedbed for Sugar Beets.—In 1935 on this same farm two fields were being prepared for sugar beets. The smaller field was nearly all Yolo clay loam; the larger field had two textural types, Yolo loam and Yolo fine sandy loam. Representative areas of each of the three types were selected, and samples were taken after each tillage operation. Volume-weight determinations were made as the samples were taken. The two fields were not worked simultaneously, and the treatments were not exactly alike. The areas of loam and fine sandy loam, however, were worked in one unit, and so these two types can be readily compared. The data for the loam texture are shown in figure 5 and those for the fine sandy loam in figure 6.

On this field, the plowing was done with moldboard plows, and a small amount of leveling was done by a large, heavy, wooden float. The disking and harrowing were done in one operation with a spike-tooth harrow attached behind a double-disk harrow. After harrowing, the field was seeded and then rolled with a cultipacker.

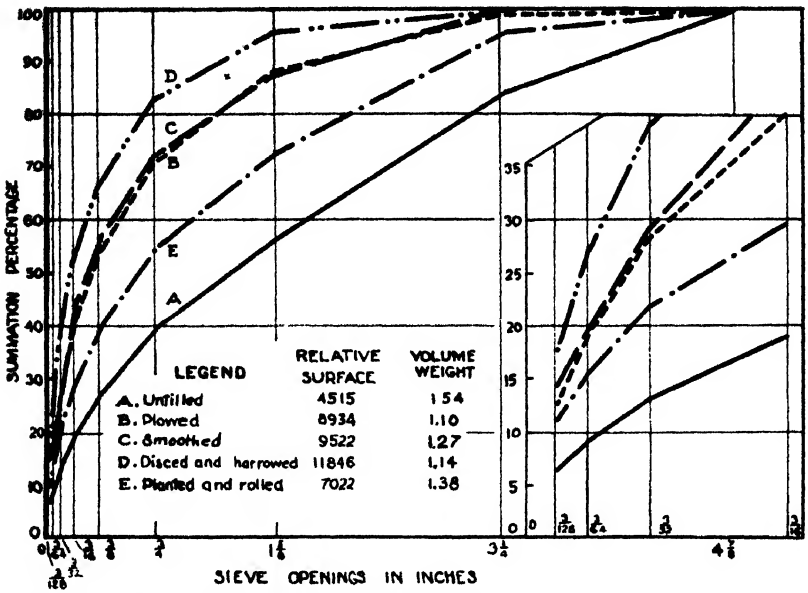

Fig. 5.—Size distribution of aggregates before and after tillage operations in preparation of seedbed for sugar beets on Yolo loam, 1935.

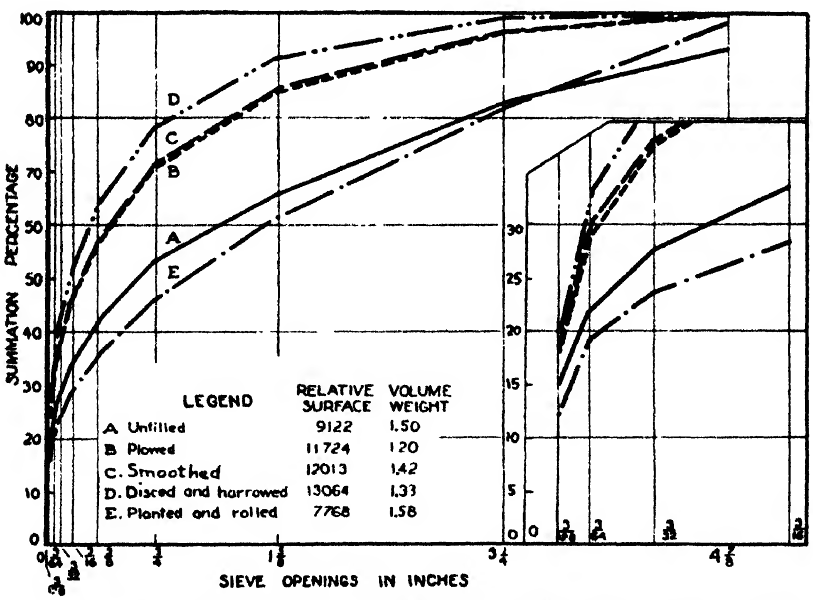

Fig. 6.—Size distribution of aggregates before and after tillage operations in preparation of seedbed for sugar beets on Yolo fine sandy loam, 1935.

On both of these types, plowing had a very pronounced pulverizing effect and also a definite loosening effect. The curves for the plowing treatment lie above those for the soil before tilling in both cases, and there was a definite decrease in the volume weight of the soil: the Yolo loam decreased in volume weight from 1.54 to 1.10 and the fine sandy loam from 1.50 to 1.20, after plowing.

Although, with both types, the relative-surface values were slightly higher after the use of the float, no appreciable change in the size distri-

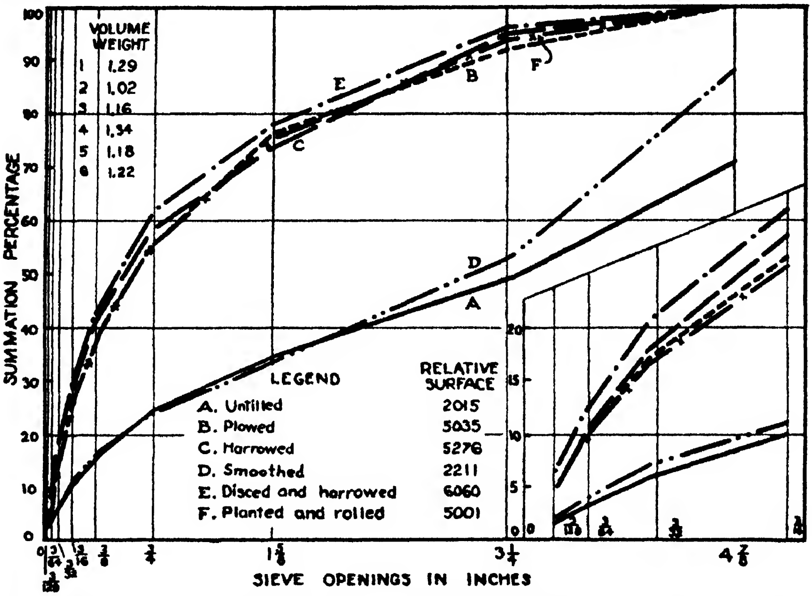

Fig. 7.—Size distribution of aggregates before and after tillage operations in preparation of seedbed for sugar beets on Yolo clay loam, 1935.

bution of aggregates occurred: curves *B* and *C* are almost identical throughout their length, both in figure 5 and in figure 6. On the other hand, the use of the float had a very pronounced effect in compacting the soil, as shown by the increases in the volume weights. On both types, the harrowing had a decided pulverizing and loosening effect. Volume weight decreased very definitely: in the case of the Yolo loam, it was reduced almost to that after plowing.

The planting and rolling in both instances increased the cloddiness and the volume weights. On the loam texture, the soil was somewhat more pulverized than before plowing, and the volume weight somewhat less. On the fine sandy loam, however, the soil was a little more cloddy and a little more compact after the final operation than before any tillage was performed.

The field of Yolo clay loam on the same farm, also being prepared for sowing sugar beets, was tilled a little later, and during the process there were a number of storms, which slightly altered the procedure on this field. Furthermore, this field was still in sod, whereas the other field on the loam and fine sandy loam types had been in a cultivated crop the year before. After plowing, this field was harrowed with a spike-tooth harrow to further break up the sod before it was worked with the float, so that this field had one more tillage operation than the other. The results on

TABLE 7

MOISTURE CONDITION OF SOILS OF THE YOLO SERIES AS TILLAGE OPERATIONS WERE PERFORMED IN PREPARATION OF SEEDBED FOR SUGAR BEETS, 1935

Treatment	Yolo loam (M.E., 20.13)		Yolo fine sandy loam (M.E., 18.78)		Yolo clay loam (M.E., 26.58)	
	Per cent H_2O	Per cent $\frac{H_2O}{M.E} \times 100$	Per cent H_2O	Per cent $\frac{H_2O}{M.E.} \times 100$	Per cent H_2O	Per cent $\frac{H_2O}{M.E.} \times 100$
Untilled	19 3	94 8	14 7	78 2	23.5	89.4
Plowed	17 3	84 8	12 8	68 1	17.6	64.3
Harrowed					20 0	75.2
Smoothed	16 4	80 5	11 0	58 5	23 1	86.9
Disked and harrowed	16 1	79 1	11 2	59 7	16.2	61.0
Rolled and planted	16.1	79 1	11 1	59 1	15 0	56.4

the size distribution of aggregates and volume weights, and the relative-surface values, are given in figure 7.

Plowing had a very marked pulverizing effect on this soil (curves *A* and *B*, fig. 7), but subsequent harrowing had no further effect, for the curves (*B* and *C*) are almost coincident throughout their length, although the relative-surface value is slightly higher after harrowing. The plowing here caused a distinct decrease in the volume weight, but the harrowing had a slight compacting effect. The subsequent use of the float increased both the cloddiness and the volume weight; in fact, the volume weight was greater than before plowing, despite the fact that the field had previously been in pasture to sheep for a number of years. The increase in cloddiness brought about by the use of the float was caused by the wetness of the field when this operation was performed. Table 7 gives the moisture conditions of the three soil types on this farm at the time of sampling.

The Yolo clay loam had been given considerable time to dry out after the use of the float, so that when the field was disked and harrowed, it was at a very favorable moisture content for tillage operations. This operation (fig. 7, curve *E*) had a distinct pulverizing effect, and the field

at this time was in its most finely pulverized condition. The planting and rolling operations increased the cloddiness only slightly. Disking and harrowing reduced the volume weight somewhat, but the subsequent planting and rolling altered it only slightly. This field, after final fitting and planting, was in a more pulverized condition than before treatment; the volume weight was only a little lower.

Seedbed for Alfalfa.—In 1934 a number of small plots were worked up for seeding alfalfa on the University Farm at Davis, California. After

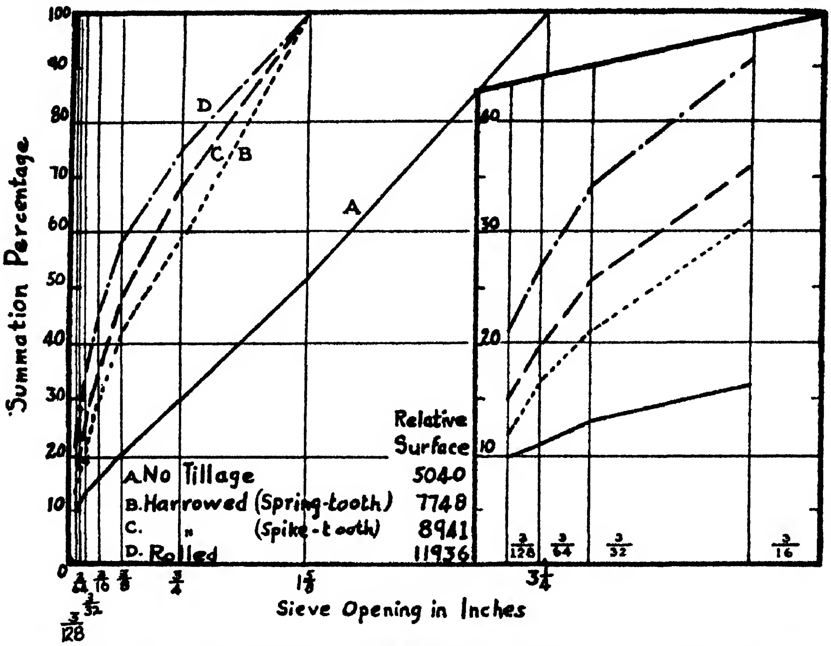

Fig. 8.—Size distribution of aggregates before and after tillage operations in preparation of seedbed for alfalfa on Yolo loam, plot D–15, 1934.

the seedbeds had all been prepared, but before they were seeded, there was a period of heavy rains. After the rains, the surface of the plots crusted over and had to be reworked. Samples were obtained from three of these plots, all on Yolo loam, as the tillage operations were being performed to reprepare the seedbeds. Since the plots here were very small, an 8-inch sampling cylinder was used instead of the 14-inch cylinder that had been used on the other samplings.

Three implements were used in working these plots for seeding. They were first worked with a spring-tooth harrow, then with a spike-tooth harrow, and finally with a spike-tooth roller. The data are plotted on figures 8, 9, and 10. Two of the three plots behaved very much alike (figs. 8 and 9). In these two plots, each tillage operation had a distinct pulver-

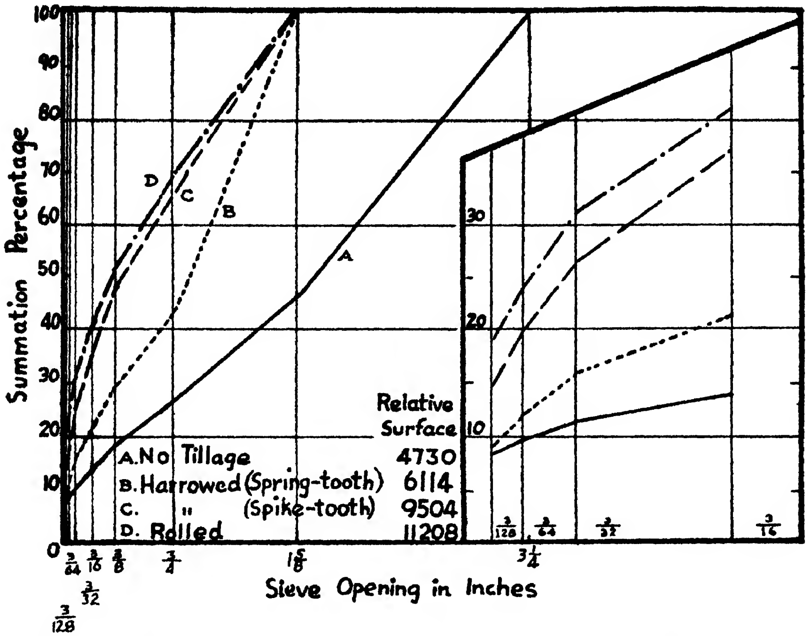

Fig. 9.—Size distribution of aggregates before and after tillage operations in preparation of seedbed for alfalfa on Yolo loam, plot D–25, 1934.

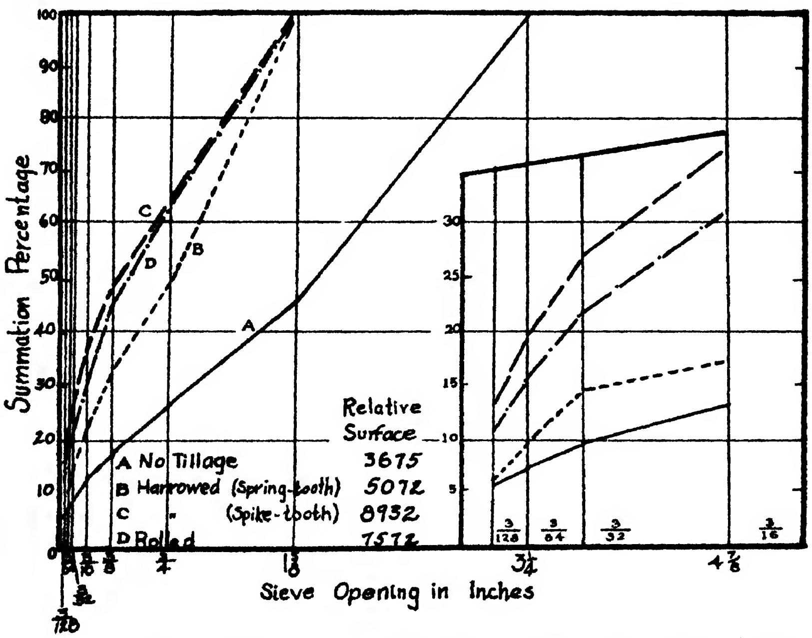

Fig. 10.—Size distribution of aggregates before and after tillage operations in preparation of seedbed for alfalfa on Yolo loam, plot D–35, 1934.

izing effect, although the intensity of the effect was somewhat different: on plot D–15 (fig. 8) the spring-tooth harrow had a greater pulverizing effect than the spike-tooth harrow, whereas the pulverizing effect for these two implements was about the same on plot D–25 (fig. 9). Plot D–35 (fig. 10) is very similar to plot D–25 in the pulverizing effect of the dif-

TABLE 8

Dates of Treatments and Sampling on Yolo Loam Plots for Special Study

	1933			1934			1935		
	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3
Plowed		3/4*	2/18*		6/7*	5/17*			
Harrowed		4/3	4/3		6/15	6/15			
Irrigated 1st series	4/11 4/14 4/17 4/20	4/11 4/14 4/17 4/20	4/11 4/14 4/17 4/20	5/1 5/2 5/3 5/4	5/1 5/2 5/3 5/4	5/1 5/2 5/3 5/4	4/30	4/30	4/30
2nd series	5/11 5/12 5/13 5/15 5/16	5/11 5/12 5/13 5/15 5/16	5/11 5/12 5/13 5/15 5/16	7/3 7/5 7/6	7/3 7/5 7/6	7/3 7/5 7/6			
3rd series	7/24 7/25 7/26	7/24 7/25 7/26	7/24 7/25 7/26						
4th series	12/1	12/1	12/1						
Sampled	3/4 5/4 12/1	3/4 4/3 5/4 12/1	3/4 4/3 5/4 12/1	5/19 7/25	5/18 6/11 6/15 7/26	5/14 6/11 6/15 7/26	4/30 6/3	4/30 6/3	4/30 6/3

* Moisture content when plowed: 1933, plot 2, 20.0 per cent; plot 3, 25.4 per cent, 1934, plot 2, 16.1 per cent; plot 3, 23.2 per cent.

ferent harrows. The rolling had a distinct pulverizing effect on plots D–15 and D–25, but the cloddiness on plot D–35 was increased somewhat by this operation. This plot was in a slight depression and had a higher moisture content when worked, which undoubtedly accounts for the increased cloddiness caused by rolling.

The relative-surface values are given with the legend on each figure. In some instances two curves may appear very close together, yet there will be fairly wide differences in their relative-surface values—compare, for example, curves and relative-surface values for spike-tooth harrowing and for rolling, in figures 9 and 10. In each case, the sample with the higher relative-surface value has the greater percentage of the finer frac-

tions. This is shown in the enlargements of this portion of the curve in the lower right-hand corner of each figure.

Tillage Operations at Different Moisture Contents.—A number of samplings were taken for size distribution of aggregate studies after plowing, harrowing, and irrigation treatments on two sets of plots on the

TABLE 9

DATES OF TREATMENTS AND SAMPLING ON YOLO SILT LOAM PLOTS FOR SPECIAL STUDY

	1933			1934			1935		
	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3
Plowed		3/4*	2/18*		6/7*	5/17*			
Harrowed		4/3	4/3		6/15	6/15			
Irrigated 1st series	4/4	4/4	4/4	5/1	5/1	5/1	5/1	5/12	5/1
	4/5	4/5	4/5	5/2	5/2	5/2			
	4/10	4/10	4/10	5/3	5/3	5/3			
				5/4	5/4	5/4			
2nd series	5/12	5/12	5/12	7/3	7/3	7/3			
	5/15	5/15	5/15	7/5	7/5	7/5			
	5/16	5/16	5/16	7/6	7/6	7/6			
3rd series	7/26	7/26	7/26						
	7/27	7/27	7/27						
	7/28	7/28	7/28						
Sampled	3/25	3/25	3/25	5/19	5/22	5/15	5/1	5/1	5/1
		4/3	4/3	7/26	6/11	6/11	6/3	6/3	6/3
	5/4	5/4	5/4		6/15	6/15			
	12/2	12/2	12/2		7/26	7/26			

* Moisture content when plowed 1933, plot 2, 20.0 per cent; plot 3, 24.5 per cent; 1934, plot 2, 17.6 per cent; plot 3, 23.2 per cent.

University Farm that were laid out for water-penetration studies as affected by tillage at different moisture contents. The moisture-penetration studies were made in coöperation with N. E. Edlefsen of the Division of Irrigation Investigations and Practice; the results of these will not be reported here. The two sets of plots were located on Yolo loam and Yolo silt loam, and there were three plots in each set. One plot received no tillage, one was plowed at what was considered a favorable moisture content for plowing, and the other was plowed at moisture contents that were considered too high to obtain the most favorable results from tillage (see footnote, tables 8 and 9). Both of the plowed plots were harrowed lightly after they had been plowed. The plots were 45 × 90 feet and were plowed with a two-horse plow in one direction only, so as not to produce a back furrow in the center of each plot.

After the tillage operations had been completed on both plots in each

set, all three plots were seeded to barley, primarily to keep out weeds. One to four series of irrigations were made on each plot (tables 8 and 9), and the rate of penetration of water into the soil was determined. After each tillage operation, the two tilled plots in each set were sampled; and after some of the series of irrigations, all of the plots in each set were sampled. Samples were taken to 7-inch depths and in 4 replicates. Each sampling removes considerable soil, so that sampling for size distribution of aggregates could not be done after each series of irrigations for fear of altering the rate of water penetration. Tables 8 and 9 give dates of treatments and sampling on these two sets of plots. During the 1934 season, because of conditions which could not be controlled, the plowing was not done until fairly late on these plots, so that before any cultural treatments were made, all plots were subjected to a series of irrigations.

The results of the samplings on these areas are given in the form of graphs similar to those previously presented. For convenience in discussing the graphs, the plots of each set are numbered as follows: plot 1 received no tillage, plot 2 was plowed at favorable moisture content, and plot 3 was plowed while too wet. Figures 11 and 12 give the results on plots 2 and 3 for the 1933 season and figures 13 and 14 for the same plots for the 1934 season.[6]

The plowing (fig. 11) had a definite pulverizing effect on plot 2, which was plowed at a favorable moisture content; but plot 3, which was plowed too wet, was more cloddy after plowing than it had been originally. The plowing in the latter case was done when the soil was too wet to scour off the plowshare. The soil seemed to push up in front of the plow and was thus compacted, which increased the cloddiness. Harrowing had no pulverizing effect on the area plowed at suitable moisture content, in fact, the curve showing the result of this treatment falls slightly below that for plowing (fig. 11, curves *B* and *C*). This slight difference is probably due to the natural heterogeneity of the soil rather than to any effect of tillage. On the plot that was plowed too wet, some pulverizing of the soil, mostly increasing the finer fraction, was caused by harrowing. The amount of coarse material is about the same, but the increase in the fine material accounts for the increase in the relative-surface value (fig. 11, curves *D* and *E*).

For the same season, on Yolo silt loam (fig. 12), the plowing had a definite pulverizing effect on both areas, but the action was more pro-

[6] In order to reduce the number of samplings on plots 2 and 3, they were not sampled before plowing, but the sampling on plot 1 taken at that time is used for these plots. Since the areas are fairly uniform and the plots are small, these results are probably very close to what would have been obtained for the other plots had they been sampled at this time.

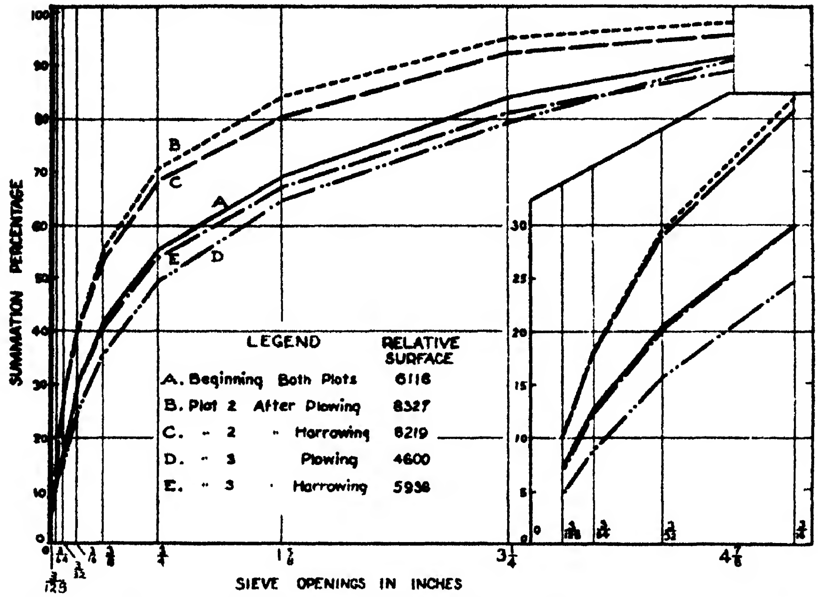

Fig. 11.—Size distribution of aggregates before and after tillage operations on plots 2 and 3 on Yolo loam, 1933.

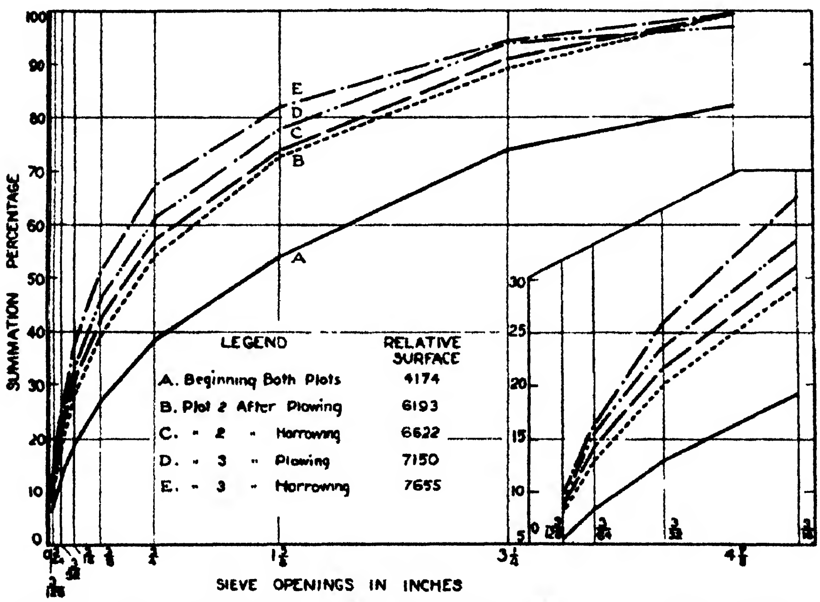

Fig. 12.—Size distribution of aggregates before and after tillage operations on plots 2 and 3 on Yolo silt loam, 1933.

nounced on the plot (No. 3) plowed at the higher moisture content. In this instance, however, although the furrow slice coming off the plowshare had a very slick shiny surface, the soil was not wet enough to prevent the soil from scouring off the plowshare.

Harrowing on these two plots seems to have had only a slight pulverizing effect. In both cases the curve for the harrowing treatment is slightly above that for plowing. There is some increase in the relative-surface values and, as shown on the enlargement of the lower end of the curves, some increase in the finer fractions.

In 1934 on Yolo loam (fig. 13), the plot that had been plowed too wet the year before was more cloddy before plowing than the one that had been plowed at a favorable moisture content. Plowing had a very definite pulverizing effect on both plots. The pulverizing effect on the wet plot was slightly greater than that on the drier one. The curves (*B* and *E*) for the plots after plowing fall closer together than those (*A* and *D*) for the plots before plowing. The numerical increases in the relative-surface values are similar for the two plots: the increase on the drier plot was from 4,718 to 7,186, an increase of 2,468; and on the wet plot from 2,936 to 5,463, an increase of 2,527.

Harrowing had a pulverizing effect on both areas (curves *C* and *F*). Here again, the amount of pulverizing on the wet plot exceeds that on the drier plot for this treatment. After both tillage operations, plot 2, the drier plot, was still in a slightly finer state of pulverization than plot 3.

For the same season, on Yolo silt loam (fig. 14), the curves (*A* and *D*) for the size distribution of aggregates before treatment fall very close together, yet there is considerable difference in the relative-surface values. Here again the large difference in this value is caused by the difference in the finest fraction. The curves are almost parallel, but curve *A* starts slightly above curve *D*.

Plowing had a distinct pulverizing effect on both plots, with a slightly greater effect on the drier plot, while harrowing had no pulverizing effect on the drier plot and only a very slight effect on the wet plot. After both tillage operations, the curve (*C*) for plot 2 is slightly higher than that (*F*) for plot 3, but the curves lie very close to each other throughout their length. The tillage operations seem to have had a very similar effect on both plots for this soil type.

In order to compare the results of treatments on the different soil types, the data were replotted. Figure 15 shows for the two soil types the results of tillage operations on the plots plowed at favorable moisture conditions in 1933.

The curves in figure 15 indicate that, when plowing was done at a

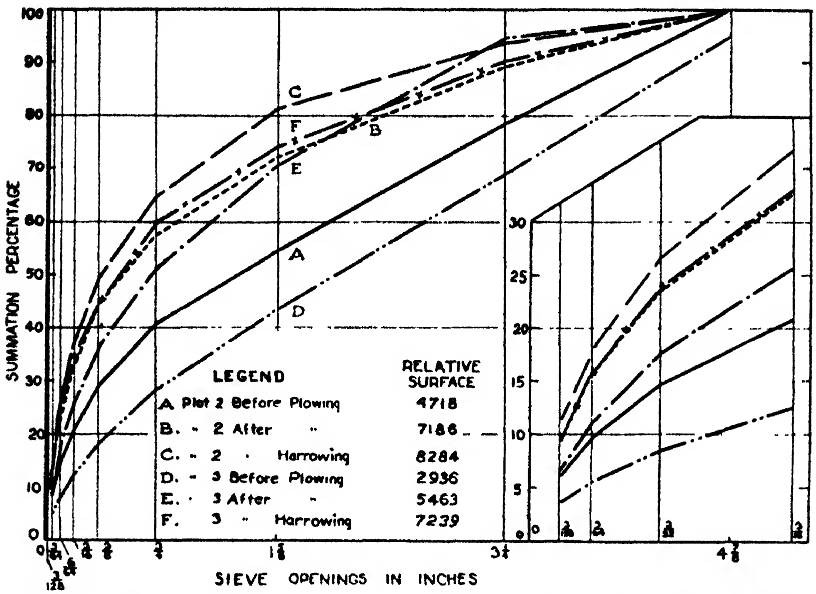

Fig. 13.—Size distribution of aggregates before and after tillage operations on plots 2 and 3 on Yolo loam, 1934.

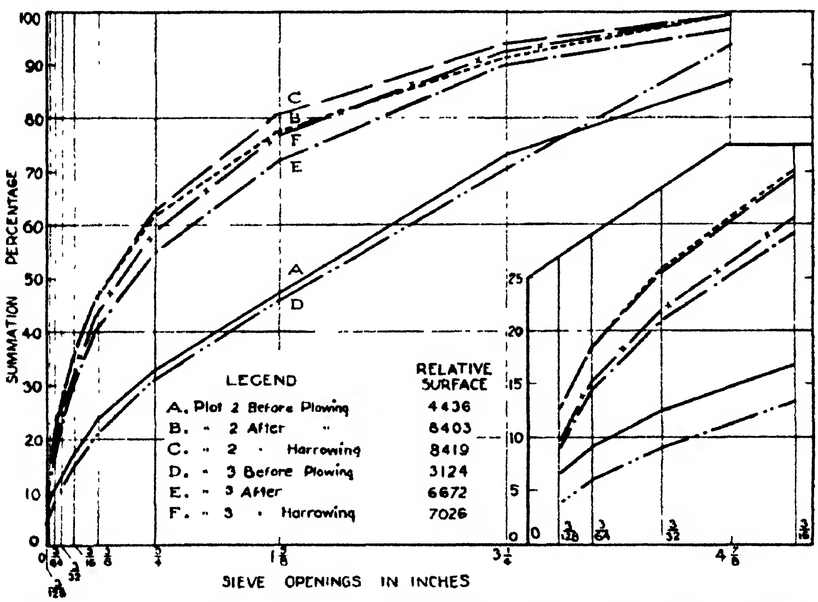

Fig. 14.—Size distribution of aggregates before and after tillage operations on plots 2 and 3 on Yolo silt loam, 1934.

favorable moisture content, the effects of tillage operations were very similar on the two soil types. The Yolo silt loam was more cloddy before any tillage and also more cloddy after the tillage. On both types, the curves for the plowing and harrowing are very close together, although on the Yolo silt loam the relative-surface value after plowing is slightly

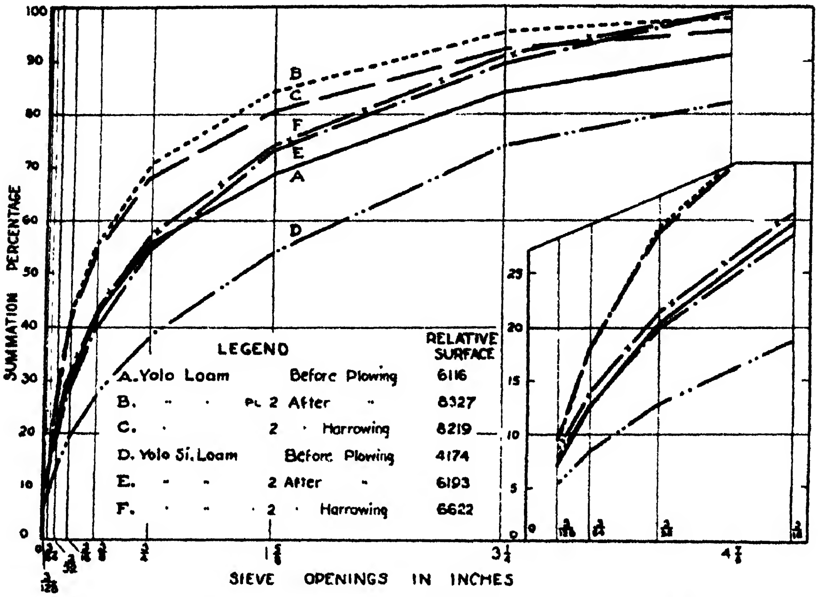

Fig. 15.—Size distribution of aggregates before and after tillage operations on plot 2 of both Yolo loam and Yolo silt loam, 1933. (Curves *A* and *D* from plot 1).

lower than after harrowing; on the Yolo loam, the relative-surface values for the two treatments are almost identical.

Similar graphs were made for plot 2 for 1934 and for plot 3 for each year, but since this constitutes merely a regrouping of the curves already given in figures 11, 12, 13, and 14, these graphs are omitted.

A comparison of curves *A, B,* and *C* on figures 13 and 14, shows that on plot 2 for 1934 on both soils, there was a stronger pulverizing effect on the silt loam, although both plots showed a very definite decrease in cloddiness. Harrowing in this year had a distinct pulverizing effect on the loam but no effect on the silt loam. After the tillage operations, these two soils were in very much the same condition (curve *C,* figs. 13 and 14).

With the plots plowed at high moisture contents, there was in 1933 a strong contrast in the effect of plowing. Before plowing, the Yolo loam was less cloddy than the silt loam (curve *A,* figs. 11 and 12), but the plowing increased the cloddiness on the loam plot and decreased it on the silt loam (curve *D,* figs. 11 and 12), so that after plowing the condi-

tion of the two plots was just reversed. Harrowing had a definite pulverizing effect on the loam and only a slight pulverizing effect on the silt loam. At the end of the tillage operations the former was nearly the same as it was originally, whereas the latter was much less cloddy.

Next year both plots that had been plowed too wet were in about the same condition before plowing, but materially more cloddy than they were the previous year. The plowing greatly reduced the cloddiness on both plots (curves *D* and *E*, figs. 13 and 14) but especially on the silt loam. The harrowing had only a slight effect on both plots (curve *F*, figs. 13 and 14) but was slightly more effective in pulverizing the loam plot, so that after the tillage operations, both plots were again very similar but much less cloddy than they had been before the tillage operations.

EFFECT OF IRRIGATION ON SIZE DISTRIBUTION OF AGGREGATES

The same two areas were sampled after some of the series of irrigations, and the size distribution of the aggregates after the irrigation may be compared with that before irrigation. The irrigation water was applied in basins to depths of about 6 inches at each application. On these plots there were from 3 to 5 applications of water, usually only a day or two apart. Figures 16 and 17 give the curves for the size distribution of aggregates for the Yolo loam and Yolo silt loam plots for 1933. In all cases there was some increase in cloddiness after the irrigation.

On the Yolo loam (fig. 16), a great increase in cloddiness occurred on the untilled check plot (No. 1) and on plot 2, which was plowed at a favorable moisture content. On plot 3, plowed too wet, there was only a slight change in the relative-surface value, yet the curves (*E* and *F*) are rather well separated in the upper half. They are, however, very close for the three finest fractions, which have the greatest influence on the relative-surface value. The peculiar behavior of this plot is further reflected in the slightness of its change after the irrigation treatment as compared to the other two plots sampled at the same period.

On the untilled Yolo silt loam, according to the curves (fig. 17, curves *A* and *B*), a considerable increase in the cloddiness was caused by irrigation. But this difference is not reflected in the relative-surface values for these two treatments: there is a slight difference, showing possibly a slight increase in cloddiness, but not nearly so much as one would expect from the comparison of the two curves. The slightly greater amount of the two finest fractions after the irrigation treatment accounts for this. Irrigation of the two plots that had been tilled caused an increase in the cloddiness. The increase was far greater, however, in the plot plowed too

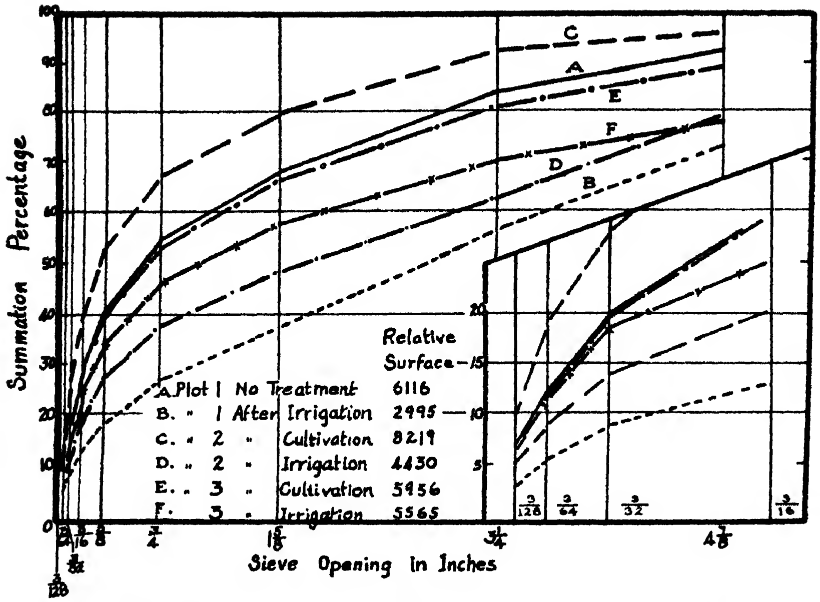

Fig. 16.—Size distribution of aggregates before and after irrigation on all three plots on Yolo loam, 1933.

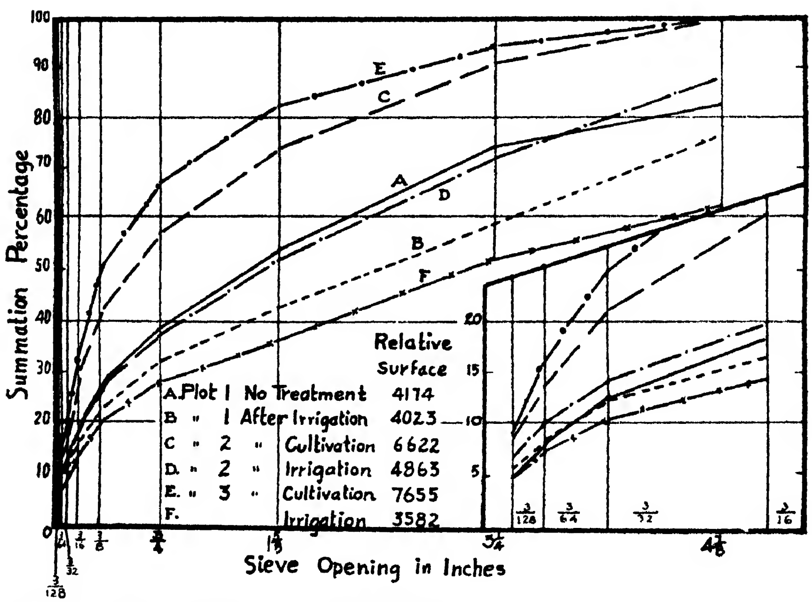

Fig. 17.—Size distribution of aggregates before and after irrigation on all three plots on Yolo silt loam, 1933.

wet (curves *E* and *F*) than in the one plowed under favorable moisture conditions (curves *C* and *D*). On these two plots, the differences in both the curves and the relative-surface value are pronounced.

Figures 18 and 19 show the results of similar treatments on the same two soil types for 1934. On the Yolo loam during this season there was a definite increase in cloddiness after the irrigation treatments on each of the plots. The change was not so pronounced for this year as it had been the previous year for the plot receiving no tillage operations. The plot plowed too wet showed a greater increase in cloddiness this season than it had the season before, whereas the plot plowed under favorable moisture conditions behaved about the same during the two seasons.

On the Yolo silt loam (fig. 19), the untilled plot showed no difference in its condition before and after the irrigation treatment and, in fact, not much difference from its condition at the beginning of these investigations in 1933. The relative-surface values before any treatments and after the irrigation treatments in 1934 are very close. There was a great increase in the cloddiness after irrigation on the other two plots on this same soil. On the plot plowed under favorable moisture conditions, the increase for this season was far greater than was the case for the previous season, whereas the behavior of the plot plowed too wet was very similar for both seasons.

After the tillage operations on each series of plots in 1933, the plots were all irrigated, then planted to barley. During the summer, two more series of irrigations were made, the last one near the end of July. The plots were permitted to remain untouched thereafter until early in December, when they were again sampled. The results are given in figures 20 and 21. There does not appear to be any consistent order of change. Some of the plots are more cloddy and some are less cloddy than at the previous sampling period.

The results on the Yolo loam (fig. 20) show a much less cloddy condition for plot 1 at the later sampling period. Curves *A* and *B* are wide apart and the relative-surface values are 2,995 and 5,728, respectively. The plot plowed at a favorable moisture content was only slightly altered (curves *C* and *D*), whereas the plot plowed at a very high moisture content was somewhat cloddier at the end of the season.

The variability between plots is not nearly so great on the Yolo silt loam (fig. 21). On this soil there was little or no difference in the aggregate condition of plot 1 at the beginning and end of the season according to the curves (*A* and *B*), and only a very slight difference according to the relative-surface values. Plot 2 was somewhat more cloddy at the later sampling period and plot 3 much less so.

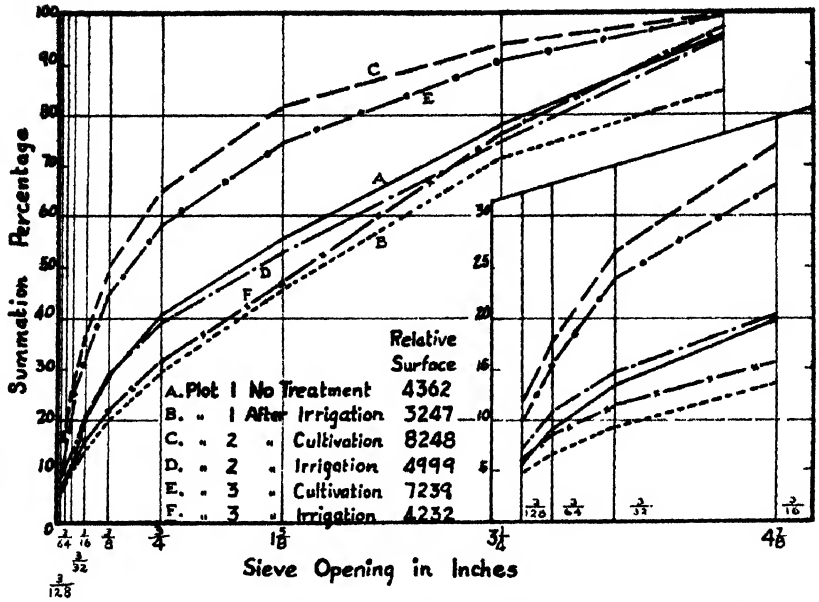

Fig. 18.—Size distribution of aggregates before and after irrigation on all three plots on Yolo loam, 1934.

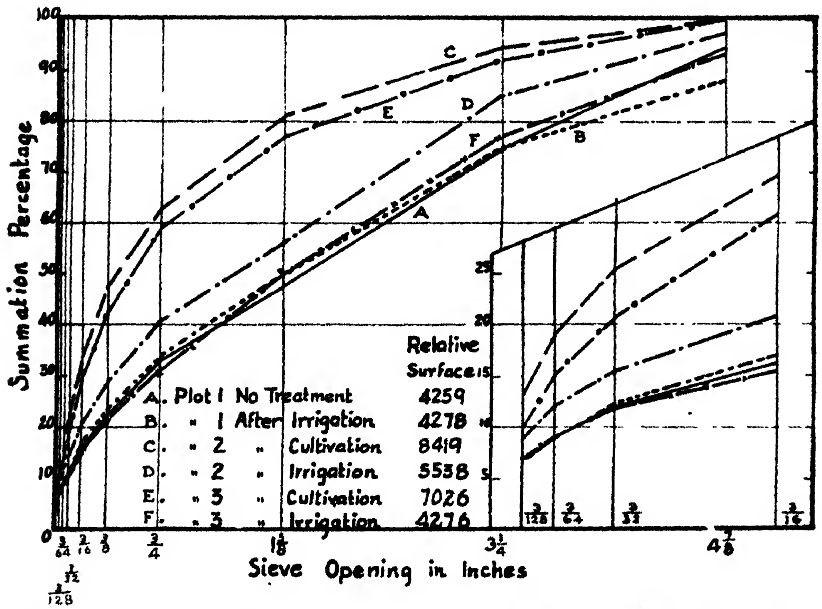

Fig. 19.—Size distribution of aggregates before and after irrigation on all three plots on Yolo silt loam, 1934.

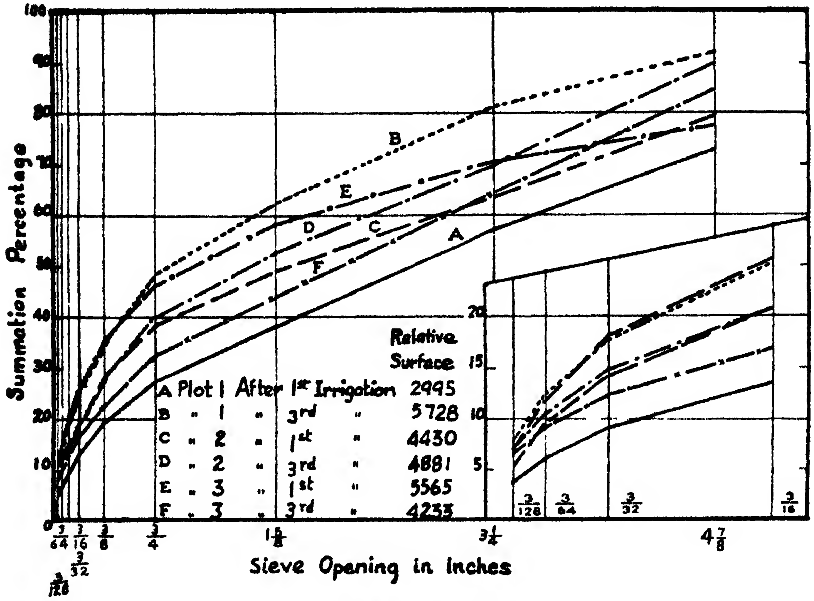

Fig. 20.—Size distribution of aggregates after the first and third irrigations on all three plots on Yolo loam, 1933.

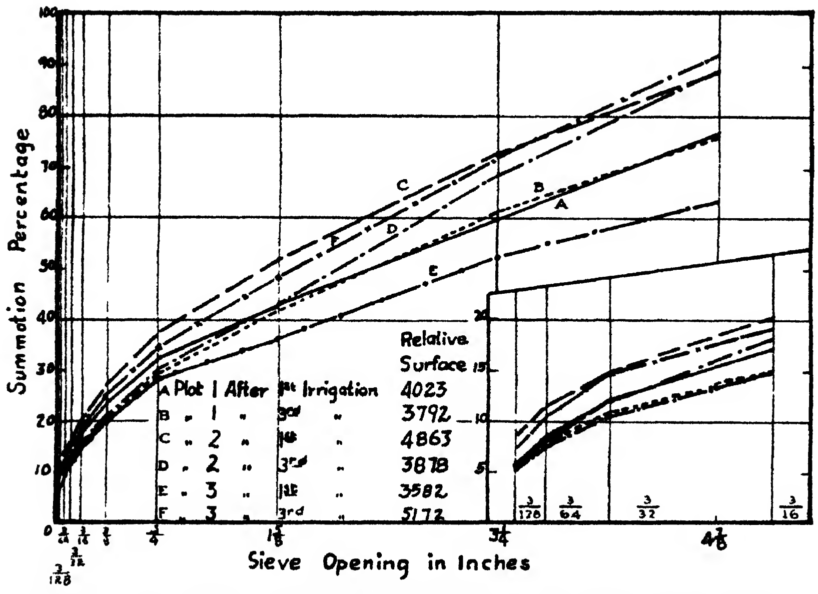

Fig. 21.—Size distribution of aggregates after the first and third irrigations on all three plots on Yolo silt loam, 1933.

The results on the two soils are directly contrasting: where there was a strong decrease in the cloddiness in plot 1 of the Yolo loam, there was practically no change for the same plot on the Yolo silt loam. On plot 2 of the Yolo loam there was a slight decrease in cloddiness and a rather definite increase on the Yolo silt loam. On plot 3 there was a definite increase in cloddiness on the Yolo loam and a slightly greater decrease on the Yolo silt loam. There does not seem to be any satisfactory explanation for the consistent contrast between these two soils in their responses to repeated irrigations. The results of the tillage operations already given were fairly consistent for both of these soils.

The effect of irrigation after tillage on the Yolo clay loam and Capay adobe clay, where the fields were being prepared for seedbed, has already been mentioned (figs. 3 and 4, p. 445). In both of these instances, as was the case with nearly all others, irrigation following cultivation caused an increase in cloddiness of the soil, so that the field, after irrigation, was more cloddy than before it was tilled.

SEASONAL VARIATIONS

The variation of the size distribution of aggregates from season to season is often as great as changes brought about by tillage operations or application of irrigation water. The seasonal changes were observed on the series of plots on Yolo loam and Yolo silt loam described in the preceding section.

In the spring of 1934 all plots were irrigated before the samples were taken, so that the changes occurring in the size distribution of aggregates over the winter period were not determined. The results for the period between the last sampling in 1934 and the first sampling in 1935 are given in figures 22, 23, and 24. In all plots on both soil types, the soils are more cloddy in the spring than they were the previous fall. The changes are greater on the Yolo silt loam than on the Yolo loam. On plots 1 and 2 (figs. 22 and 23) at the last sampling in 1934, the Yolo loam was somewhat coarser than the Yolo silt loam, and on plot 3 (fig. 24), the two soils had about the same size distribution of aggregates. When samplings were taken the following spring, plot 1 on the Yolo loam, according to its relative-surface value, was slightly coarser than the same plot on the Yolo silt loam, although, except for a short distance at the finer end, the curve (*B*) of size distribution for Yolo loam lies above that (*D*) for Yolo silt loam. On the other two plots, the Yolo silt loam is slightly coarser than the Yolo loam.

There is a great increase in the cloddiness of all plots at the conclusion of the sampling period in the spring of 1935 over that at the beginning

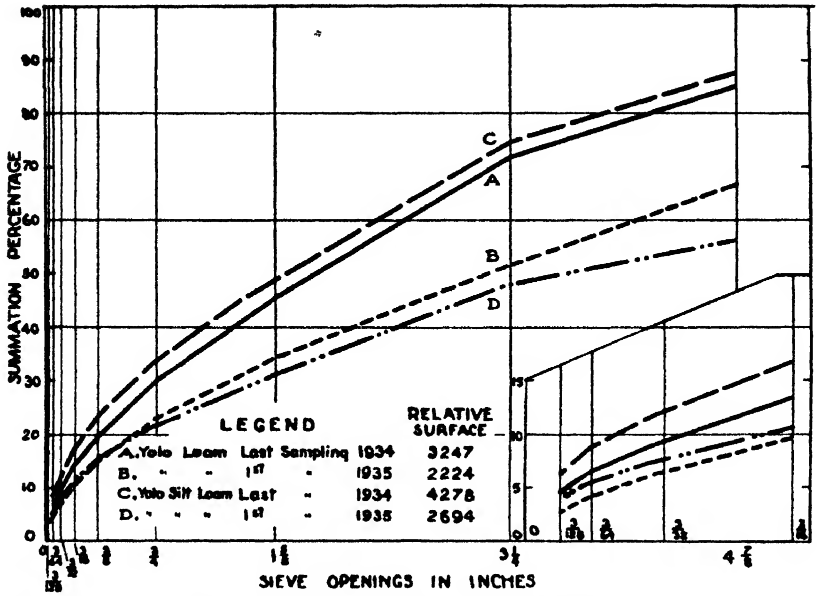

Fig. 22.—Changes in size distribution of aggregates on plot 1 of Yolo-loam and Yolo-silt-loam areas during the winter 1934–35.

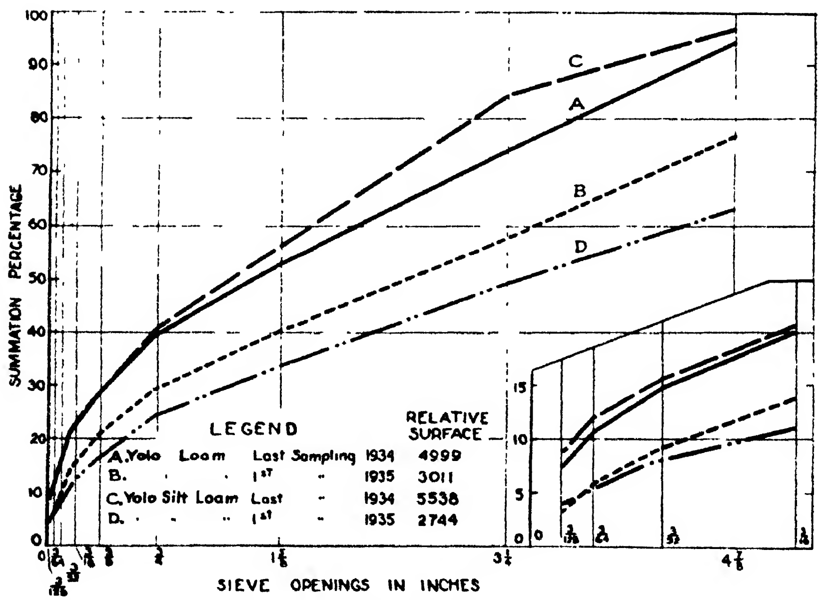

Fig. 23.—Changes in size distribution of aggregates on plot 2 of Yolo-loam and Yolo-silt-loam areas during the winter 1934–35.

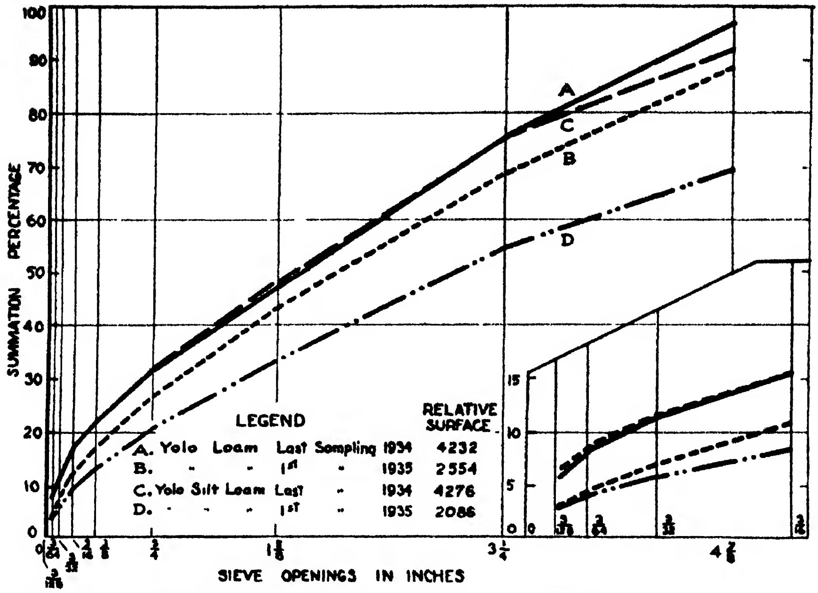

Fig. 24.—Changes in size distribution of aggregates on plot 3 of Yolo-loam and Yolo-silt-loam areas during the winter 1934–35.

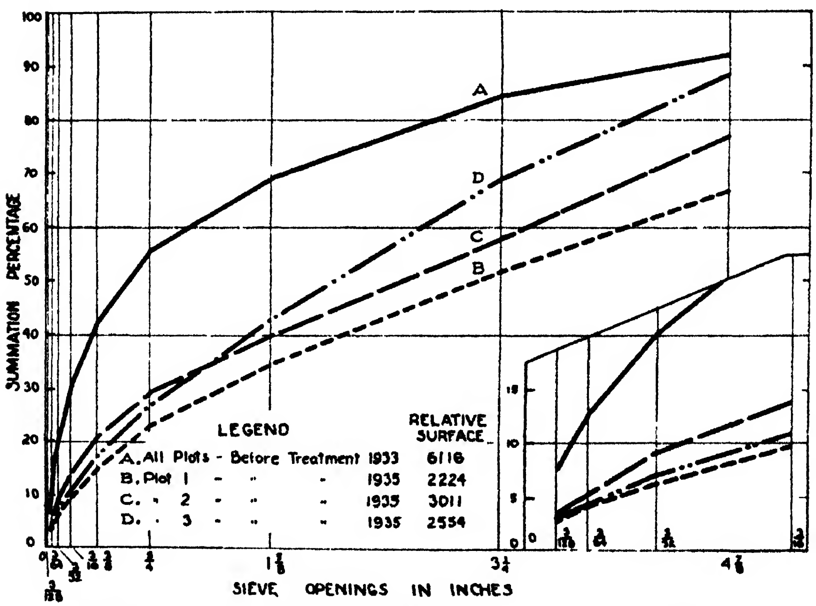

Fig. 25.—Size distribution of aggregates on all plots on Yolo loam in springs of 1933 and 1935.

of the experiment in 1933. The data for these sampling periods are plotted in figures 25 and 26. The tillage operations did not have a lasting effect on the size distribution of aggregates on either soil; for the untilled plots had as much change as did the tilled plots. In fact, in 1935 on the Yolo loam, the untilled plot had the lowest relative-surface value of the

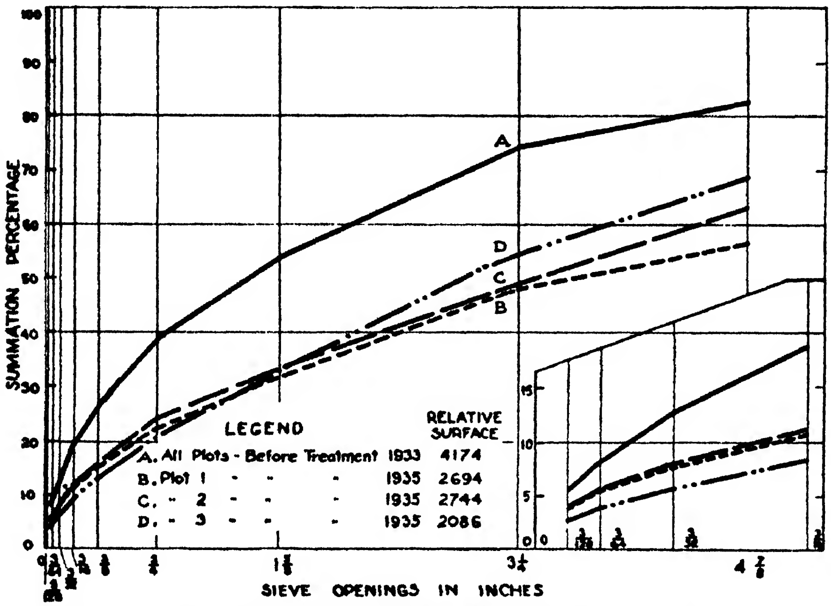

Fig. 26.—Size distribution of aggregates on all plots on Yolo silt loam in springs of 1933 and 1935.

three plots, whereas on the Yolo silt loam, the plot plowed too wet had the lowest value.

The seasonal changes over the winter period in the vicinity of Davis are somewhat similar to those obtained by irrigation treatments. This is not surprising because the winters at Davis are mild, the soils never freeze, and nearly all of the season's total rainfall of about 17 inches comes in a five-month period from December to May. The surface soils are usually wet to field capacity after the first few rains and remain so until the end of the rainy season, with seldom an opportunity for drying out.

DISCUSSION

From the data presented, and the rather consistent general tendencies observed, the air-dry sifting used throughout this work appears to give a better picture of the size distribution of aggregates in the field than when the samples are sifted moist, despite the fact that the replicate samplings are equally variable by either method. The aggregates in the

moist condition are so weak that the mechanical action necessary for a good separation into the respective sizes is sufficient to break down some of them, so that the resultant data do not give an accurate picture of the size distribution at the time of sampling.

It is fairly certain that the method of sifting in the air-dry condition gives a good picture of size distribution just before sifting, but the question still arises as to whether or not the samples when air-dry and ready for sifting had the same condition of aggregation as that when in the field prior to sampling. The method of sampling being uniform, the change, if any, in the size distribution of aggregates must be very small because of the care with which the samples were taken. If a change in the size distribution of aggregates is brought about by air-drying the samples, then a further question arises as to how much difference there is in the size distribution of aggregates when the sample is dried in the boxes, as was the case in this work, and when drying takes place naturally in the field. These questions need further study, but at present it seems that the changes that take place during the drying process were not very great, and probably much smaller than the natural differences due to heterogeneity of the soil, so that the measurements of aggregates in the air-dry condition give a good picture of the size distribution of aggregates at the time the samples were taken.

The number of tillage operations used in preparing a seedbed is often more a matter of habitual practice than a careful consideration of the physical condition to be attained for the particular crop. Year after year, many farmers use the same tillage operations, irrespective of the physical condition of the soil. Under such practices, undoubtedly many tillage operations are performed that are wholly unnecessary. The farmer may just as well be saved the cost of performing such operations by a more careful observation of the condition of the field and the condition required for the use to which the field is to be put.

A consideration of the curves showing changes in size distribution of aggregates in preparing seedbeds shows that in a number of instances, the size distribution of the aggregates at the final fitting of the field for seeding was not greatly different from that before any tillage operations were performed. A rather striking example of such a condition is shown in the area of Yolo clay loam prepared as a seedbed for beans (fig. 3, curves *A* and *F*, p. 445), in which the size distribution of aggregates is almost identical before any tillage operations and after the final tillage operation.

It is a common practice, when beans and other late-planted crops are grown under irrigation, to plow and harrow, then irrigate before final

fitting for the seedbed. In view of the information regarding the effect of irrigation following tillage operations, it seems that in many instances, at least one tillage operation may be eliminated by applying water before plowing, or at least immediately after plowing, rather than after harrowing, provided the field does not need leveling, which should always be done when the soil is fairly dry.

Because of the great influence of irrigation water in increasing the cloddiness of soils that are finely pulverized, a field soon to be irrigated should not be worked down too fine. A similar rule should be followed for fields to be left over the winter. If the field is worked up too fine, the first rains will pack and puddle the surface, and prevent later rains from penetrating so easily. Not only may this cause the removal of some of the rain water that otherwise would have been absorbed, but also the runoff may cause damage by erosion. It is especially important that fall-sown crops, fallow land, and cultivated permanent crops be left loose and open and somewhat cloddy at the beginning of the rainy season. This, of course, applies only to medium- or heavy-textured soils. Leaving light-textured soils worked up too loose may cause damage by wind movement.

In all cases where irrigation water was applied after tillage, the soil was much more cloddy than before irrigation, and in many cases it was more cloddy than before tillage. In fact, in more than half of the instances here recorded, the greater the state of pulverization before irrigation, the coarser the condition after irrigation. All irrigations considered in this work were of the basin type, where the whole surface was covered with water.

These findings would indicate that where areas are to be irrigated during the summer by the basin method—which is a common practice in deciduous orchards in the flatter valley lands of California—it is not necessary to cultivate to a fine mulch before irrigation, for this will nullify all of the pulverizing effect of tillage. Since a mulching does not conserve the field moisture by reducing evaporation where the water table is more than 6 feet from the surface (*17, 19*), it seems that the only reason for cultivating in orchards under the above conditions would be for the purpose of controlling weeds, or turning under covercrops, or putting the surface soil in a condition to reduce the loss of moisture by runoff during the rainy season.

The samples from untilled plots of the Yolo loam and Yolo silt loam soils indicate that the seasonal changes in the size distribution of aggregates are great, and that the wetting of the soil, both by winter rains and by irrigation water, brings about changes in the size distribution of aggregates that are often as great as changes brought about by tillage implements.

SUMMARY

The present study offers a method of measuring quantitatively changes in the size distribution of aggregates and the volume weight of the soil.

Three factors which are important in these changes are tillage, irrigation, and winter rains. In general, the results of these factors are as follows:

1. Plowing caused a decrease in cloddiness and volume weight unless performed at excessive moisture contents.

2. Harrowing usually did some breaking up of clods. Disk and spring-tooth harrows seemed more effective than spike-tooth harrows in reducing cloddiness.

3. Rolling and leveling operations increased the volume weight and had a pulverizing effect on the very dry soils, but moderately moist soils usually showed increased cloddiness after these operations.

4. The cloddiness of soils worked up to a fine, highly pulverized condition was greatly increased by irrigation, often to a point far in excess of that before any tillage. Tilled areas that were left cloddy showed some increase in cloddiness, but not so much as those that had been highly pulverized.

5. Winter rains often have as great an influence on the size distribution of aggregates as the tillage operations or irrigations.

A general view of the data presented shows that even under fairly well controlled conditions, the results obtained by any one cultural treatment are not always the same, but fairly comparable. The differences may be due to the natural heterogeneity of the soil or to factors that could not be readily observed.

This method offers a good means of studying the effects of various tillage implements in pulverizing the soil, and from these studies, it should be possible to determine the most efficient method of preparing the soil for any desired use. Other important relations, such as seasonal changes and changes due to irrigation and subsequent drying, may be studied, which should lead to a more efficient use of irrigation water and tillage implements.

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FACTORS AFFECTING THE RECOVERY OF HYDROCYANIC ACID FROM FUMIGATED CITRUS TISSUES[1,2]

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UNDER CERTAIN conditions of fumigation for scale insects of citrus trees, hydrocyanic acid (HCN) is known to cause injury to the foliage and fruit. The physiological reactions involved in the injury have received but slight quantitative investigation. Undoubtedly the reasons for this have been the difficulties encountered in distilling HCN from materials containing volatile substances which are reactive with HCN. The lack of a method with sufficient accuracy to recover relatively small amounts of HCN from the tissues may also have been an important factor.

The results reported in this paper are concerned only with the factors affecting the distillation and recovery of HCN from solutions in the absence of tissues and also in the presence of citrus foliage and fruits. Earlier attempts to recover HCN from fumigated citrus tissues by aspiration, partial vacuum, immersing and shaking the tissues in an alkaline solution, or by a combination of these processes have given unsatisfactory results. All statements in this paper concerning the recovery of HCN from citrus tissues refer to HCN added by fumigation or by other methods. Repeated tests by Bartholomew and Raby (*3*) and in the present investigation have shown that citrus tissues do not contain autogenous HCN.

An investigation is being made of the physiological effects of HCN in citrus tissues.

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THE QUANTITATIVE DETERMINATION OF HCN IN THE ABSENCE OF CITRUS TISSUES

A modified Liebig silver-nitrate–volumetric method was employed for the determination of HCN, the end points of titration being determined by the use of a photoelectric turbidimeter. The operations were conducted according to the recommendations made by Bartholomew and Raby (*2,3*). Preliminary experiments, however, demonstrated that a new method of recovery of HCN must be developed or an old one be adapted to the material to be investigated.

Considerable difficulty was experienced in weighing accurately such small amounts of liquid HCN as were being used. The first method was

TABLE 1

Amounts of HCN Recovered from Known Quantities of NaCN Dissolved in 0.1 N NaOH Solutions

Experiment No.	Purity of NaCN per cent	NaCN added, mg	HCN equivalent, mg	HCN recovered	
				Amount, mg	Per cent
130	95.5	58.9	31 0	30.5	98 4
131	96.5	58 3	31 0	30.9	99.7
132	96.5	60.0	31.8	31.8	100 0
133	96.5	30 0	15.9	16.0	100.6

to seal 30 to 40 mg of liquid HCN in a weighed 5-ml glass ampoule. When the ampoule was broken in a liter of 0.1 N NaOH and 150-ml aliquots titrated with standard $AgNO_3$, the total amount of HCN determined was 104 to 106 per cent of the quantity originally weighed. The possible sources for this error were in the method of titration and in the method of weighing the liquid HCN. The results recorded in table 1, which show the recovery of HCN from NaCN, demonstrated that the titration method was accurate.

This left as the only other source of error, the inaccurate method of weighing: if a small amount of liquid HCN (30–40 mg) is placed in a weighed 5-ml ampoule and a second weighing is made, the difference between the two weights does not show the exact amount of HCN in the ampoule because a certain amount of the air is replaced by HCN vapor, which is lighter.[6] The actual amount of air displacement in the larger

[6] For example, at 23° C and 750-mm pressure and with a difference of 31 mg between the first and second weighings, the ampoule (5-ml capacity) would contain an excess of 19 per cent of HCN if all the air (5.9 mg) were displaced. Liquid HCN has a low boiling point—26°—and an exceedingly high vapor pressure at ordinary laboratory temperatures—654.4 mm at 22° (*9*).

containers could not be governed or determined; therefore the amount of HCN existing in the vapor phase was reduced to a minimum by using ampoules with a volume just equivalent to, or only slightly greater than, the amount of liquid HCN desired for a given test. This eliminated the difficulty.

The quantities of HCN used in the experiments were small, never over 100 mg, the average amount being about 39 mg. The laboratory fumigation chamber has a capacity of 5 liters, and the total volume of the solutions used for distillation was 3 liters. As shown by these volumes, the actual concentrations of HCN used in the experiments were very low. Before the experimental methods could be applied to citrus tissues, the conditions for the distillation and recovery of pure samples of HCN in such concentrations had to be standardized.

TABLE 2

AMOUNTS OF HCN RECOVERED AFTER VARIOUS TREATMENTS

Treatment (liquid HCN used)	Experiment No.	HCN added, mg	HCN recovered	
			Amount, mg	Per cent
A, In empty fumigation flask 45 min., then acidulated H_2O and $CdSO_4$ added	163	41 6	41 2	99.0
	165	23 5	23 5	100 0
	166	33 9	33 9	100 0
	167	11 0	11 0	100 0
B, In 0.1*N* NaOH without distillation . .	146	897 0	901 0	100 3
	147	16 7	17 0	101 8
	148	22 0	21 8	99.1
	149	44 2	44 4	100.5
	150	28 6	29 3	102 4
	153	53 2	53.5	100 6
C, In 0.1*N* NaOH with double distillation, acidulated H_2O and $CdSO_4$ added	218	33 7	33 5	99 4
	219	26 1	25 8	98 9

Having solved the difficulties previously encountered in weighing known amounts of HCN, the first step in standardizing the methods for its recovery was to break the ampoule of liquid HCN in the empty fumigation flask and allow it to remain for 45 minutes. Thirty grams of $CdSO_4$ were added and enough H_2SO_4 in each case to make the solution slightly acid—0.15 or 0.30 ml beyond neutrality (p. 477), according to the nature of the solution to be distilled. (The reason for adding the $CdSO_4$ will be explained in the following section.) The results of these tests demonstrated that no losses occurred through the apparatus connections. The tabulated results are shown as treatment A in table 2.

The second step was to break ampoules containing known amounts of

liquid HCN in the bottom of a tall cylinder containing about 900 ml of 0.1 *N* NaOH. The solution was then poured into a liter volumetric flask and diluted to volume. The solution was not distilled; therefore no acid or $CdSO_4$ was added. Aliquot samples of 150 ml each were titrated with standard $AgNO_3$. The amounts recovered were in agreement with the available amount in each sample, as shown for treatment B in table 2.

The third step was to attempt to recover the HCN from 0.1 *N* NaOH by double distillation, after having added the usual amounts of H_2SO_4 and $CdSO_4$. The first distillates were redistilled because, as explained elsewhere in this paper, the first distillate from fumigated leaves contained a volatile substance which interfered with the titration of the HCN. The data (treatment C in table 2) show that the double distillation process resulted in only a very slightly lower percentage recovery of HCN than treatment B.

THE RECOVERY OF HCN FROM CITRUS-LEAF DISTILLATES

General Methods with Leaves.—Mature or young citrus leaves were picked from the trees, placed in a container with a tight cover, and immediately brought to the laboratory and thoroughly mixed. About 15 to 20 minutes elapsed between picking the leaves and placing them in the fumigation flask. The moisture in each lot of leaves was determined on a 100-gram sample by heating the leaves at 100° C for 1 hour and then to constant weight at 70°. The loss in weight was recorded as the percentage of moisture.

Unless otherwise stated, 200 grams of mature leaves were used for each fumigation experiment. The 200-gram sample was fumigated in a 5-liter Pyrex flask and the length of the fumigation period was always 45 minutes unless otherwise stated. Pyrex flasks were used in place of ordinary soda flasks because sodium cyanide would have been formed on the walls of the latter (*9*). A small ampoule containing a known amount of HCN was broken in the flask containing the leaves. The flask was closed with a rubber stopper containing a large glass tube and a separatory funnel, both of which contained stopcocks so that an airtight seal could be made and no appreciable loss of HCN would occur. Tin foil was firmly cemented to the lower surface and sides of the stopper so that no reaction could take place between the rubber and the HCN (*5*). The flask was kept in an inverted position and shaken several times during the fumigation period.

At the end of the fumigation period, the flask was connected to a condenser by means of the large glass tube. The end of the condenser extended to the bottom of a 1-liter volumetric flask containing 100 ml of

N NaOH. The large stopcock in the tube leading to the condenser was opened, and 3 liters of acidulated distilled water was added through the separatory funnel. Enough material was distilled over to make 1 liter in the receiving flask; this brought the distillate to approximately 0.1 *N* with respect to NaOH. After the distillate had been filtered, 2 liters of acidulated distilled water and 30 grams of $CdSO_4$ were added, and another liter distilled and caught as before. Aliquots of 150 ml each of the second distillate were titrated to turbidity with standard $AgNO_3$. The concentration of the $AgNO_3$ was usually 0.020 *N*, but ranged from 0.019 to 0.022 *N*.

The amount of acid placed in the distillation flask depended on whether it was added directly to the fumigated tissues or to the alkaline distillate. In the earlier tests, 0.2666 *N* (2 per cent) tartaric acid or 0.0036 *N* H_2SO_4 (0.30 ml of concentrated H_2SO_4 in 3 liters of solution) beyond the calculated neutrality (of solutions) was used. Before the investigation had progressed very far, citrus tissues were found to be sufficiently acid in reaction to make possible the recovery of the HCN during the distillation process. Therefore during the later tests on the leaves and on all of the fruits, only 0.0018 *N* H_2SO_4 (0.15 ml in 3 liters of solution) was added. This precaution was taken to make sure that the medium would be acid in all cases.

Interference of Hydrogen Sulfide.—Difficulties were encountered at once when attempts were made to determine HCN in the distillate from fumigated leaves. The first experiments showed that the distillate contained volatile substances which were carried over with the steam and which produced a darkening of the distillate on the addition of the first increment of $AgNO_3$. Continued darkening of the solutions with further additions of $AgNO_3$ made it obvious that HCN could not be determined in the presence of such impurities. Therefore the immediate problem was to free the distillate of the volatile substances which reacted with $AgNO_3$.

There are from 2 to 4 ml of volatile oils in a liter of distillate from a 200-gram sample of leaves. The removal of most of the oil by filtration did not eliminate the titration difficulties. Quantitative tests showed that the distillate was free of organic sulfur and volatile nitrogenous compounds. The solution, however, had a slight odor of H_2S, the actual presence of which was confirmed by testing with lead acetate.

A quantitative determination of the H_2S was made by the $CdSO_4$ method, as described by Scott (*8*). A 200-gram sample of leaves was distilled and four successive portions of 200 ml each were caught in 50 ml of a 6 per cent solution of $CdSO_4$. Separate similar distillations were made with the whole fruit, peel, and pulp. As shown in table 3, the total amount

of H_2S which occurred in the distillate from the leaves or fruit was relatively small. However, when an aliquot portion was titrated with $AgNO_3$, the quantity of Ag_2S formed was sufficient to discolor the solution to such an extent that a false end point was obtained.

The problem at hand was to convert the volatile H_2S to a compound which was neither volatile with steam nor reactive with HCN. In an effort to do this, $CdSO_4$ was added just before distillation began. The H_2S reacted with the $CdSO_4$ to form CdS, which is nonvolatile and which remained in the flask during distillation. To convert all the H_2S to CdS,

TABLE 3

AMOUNTS OF VOLATILE H_2S OBTAINED FROM THE DISTILLATES OF CITRUS LEAVES AND GREEN FRUITS

Fraction (distillate) No., 200 ml each	H_2S from 200-gm samples of leaves, mg				H_2S from 15 green Valencia oranges, mg		
	Sample I	Sample II	Sample III	Sample IV	Whole fruit	Peel	Pulp
1	2 28	2 38	2.20	2 29	3 40	1 32	1 41
2	0 73	0 82	0 64	0 64	0 75	0 99	0 58
3	0 55	0 55	0 46	0.55	0.66	0 66	0 58
4	0 46		0 55	0 46	0.75	0 50	0 66
Total	4 02	3 75	3 85	3 94	5 56	3.47	3 23

however, the solution would have had to cover the leaves entirely during distillation. This was impossible because the specific gravity of the fresh leaves was less than that of the solution; hence, the $CdSO_4$, instead of being placed in the flask with the leaves, was placed in the leaf distillate, and this solution was redistilled. The double distillation process freed the solution of all H_2S.

Before applying this procedure to leaves, it was necessary to determine experimentally what effect, if any, $CdSO_4$ would have on the recovery of known amounts of HCN. This was done by breaking ampoules containing known amounts of HCN, in 0.1 *N* NaOH. This solution was then poured into the distilling flask and diluted to 3 liters. In some of the experiments H_2SO_4, and in others tartaric acid, was used. Each distillation was made in the presence of 30 grams of $CdSO_4$. The results given in table 4 show that the presence of the $CdSO_4$ did not materially reduce the percentage of recovery of HCN, and that the recoveries were the same with H_2SO_4 and with tartaric acid.

Penetration of HCN into Leaf Tissues.—A series of experiments was carried out to determine the amount of HCN that actually penetrated the leaves during fumigation. At the end of the fumigation period, the

leaves were washed in the 5-liter fumigation flask with 2 liters of 0.1 *N* NaOH by shaking thoroughly so as to get the alkaline solution to contact the walls of the flask and the surface of the leaves. The leaves were then washed four times with distilled water, 2 liters each time, and finally with 2 liters of acidulated water (containing 0.15 ml of concentrated H_2SO_4). The acid solution was poured from the leaves, then 3 liters of the acidulated water was added to the leaves in the flask and distillation conducted in the usual manner.

TABLE 4

AMOUNTS OF HCN RECOVERED AFTER DISTILLATION FROM SOLUTIONS ACIDIFIED WITH H_2SO_4 OR TARTARIC ACID

(Each solution contained 30 grams $CdSO_4$)

Acid used	Experiment No.	HCN added, mg	HCN recovered	
			Amount, mg	Per cent
H_2SO_4	151	26 1	26 2	100 4
	152	38 9	39 0	100 3
	155	34 3	34 5	100 6
	154	29 6	30 1	101 7
	161	28 8	28 7	99 7
	Average			100 5
Tartaric acid	156	53 4	53 6	100 4
	157	18 4	18 6	101 1
	158	59 9	60 0	100 2
	160	32 3	32 5	100 6
	Average			100 6

As may be seen in table 7 (p. 481) an average recovery of 66.3 per cent of the HCN added was obtained from the different lots of leaves which had received such a washing treatment in the interval between fumigation and distillation.

Retention and Fixing of HCN by the Tissues.—Another experiment was made to determine how much available HCN had remained in the leaves at given intervals after fumigation. The leaves were treated and recoveries made according to the procedure already described. At the end of the 45-minute fumigation period, the leaves were removed from the chamber, spread on ¼-inch-mesh wire screen and exposed to laboratory air for 1 minute, and for periods of 2, 4, 8, and 15 hours before they were distilled for the determination of HCN.

The results of these experiments are shown in table 5. A measurable amount of HCN remained in the leaves even after an exposure of 15 hours to the laboratory air. The HCN that could not be recovered was apparently fixed in the vapor phase or by some nonvolatile substance in the leaf tissues either before or during distillation.

In order to determine whether the reactions occurred entirely or at least largely in the vapor phases, known amounts of HCN were added to distillates from unfumigated leaves and distilled at once in the usual manner. The results of these tests, given in table 6, show an average recovery of 99.0 per cent of the HCN.

TABLE 5

AMOUNTS OF HCN RECOVERED FROM FIVE 200-GRAM LOTS OF CITRUS LEAVES EXPOSED TO LABORATORY AIR FOR DIFFERENT LENGTHS OF TIME AFTER FUMIGATION

Time aerated	Experiment No.	Moisture in leaves, per cent	HCN added, mg	HCN recovered	
				Amount, mg	Per cent
1 minute	237	56 2	48.2	29 1	60 4
	238	60.1	34.7	23.0	66 3
	241	57 0	38 7	28.3	73 1
	242	55 7	49.6	33.5	67.5
	Average	.			66.8
2 hours	222	59 5	47.3	17 5	37.0
	223	58 3	33 0	13 1	39 7
	225	59.0	44.0	16.8	38 2
	227	56 6	37 2	14 6	39 2
	Average				38.5
4 hours .	251	54 8	28 4	7 8	27.5
	252	54.8	28 9	8 6	29.8
	264	59 1	31.2	8 3	26.6
	265	59 1	27.8	7.1	25 5
	Average				27 4
8 hours	266	58 0	40 2	6 3	15.7
	267	58 0	34 2	4 6	13 5
	268	59.0	32 0	4.4	13 8
	269	59.0	43 0	6.3	14 7
	Average				14.4
15 hours...	230	58.5	37 2	7 6	20.4
	231	57.9	28 1	4 7	16 7
	239	57 3	31 2	4.2	13 5
	240	60.4	44 0	4 1	9 3
	Average				15 0

The next point was to determine whether additional HCN would react and combine with nonvolatile substances in the leaf tissues or with substances that were not volatile enough to be carried over during the first distillation. (Previous tests had shown that additional amounts of volatile substances were driven over when leaf tissues were distilled a second time.) For this purpose, known amounts of HCN were added to different 200-gram lots of unfumigated leaves and distilled at once. The results of these tests, also given in table 6, show an average recovery of only 95.8 per cent of the HCN.

TABLE 6

RECOVERY OF HCN THAT HAD BEEN ADDED DIRECTLY TO THE DISTILLATES FROM CITRUS LEAVES AND THEN DISTILLED OR THAT HAD BEEN DISTILLED IN THE PRESENCE OF MATURE CITRUS LEAVES

Treatment	Experiment No.	Moisture of leaves, per cent	HCN added, mg	HCN recovered	
				Amount, mg	Per cent
HCN added to leaf distillate	255	58.5	41.7	40 9	98.1
	256	57 5	35.1	34.7	98.9
	257	57 5	53 6	53.1	99.1
	258	57.5	25.7	25.7	100.0
	Average	..	.		99.0
HCN distilled in presence of leaves....	259	57.6	45 0	43.8	97.3
	260	57.6	34.8	33.0	94.8
	261	59.5	41 2	39.0	94.7
	262	59.5	19.5	18.8	96 4
	263	57.0	25.8	24.7	95.7
	Average	....	...	.. .	95.8

Tests (see table 2) had shown that the HCN had not escaped from the flask; therefore it appeared that either the tissues were not finely enough divided to permit all of the HCN to escape during the process of distillation, or a certain amount of the HCN was chemically bound or changed within the tissues during the periods of fumigation and distillation. An attempt to solve this problem was made by grinding 200-gram lots of fresh leaves to pass a 2-mm screen in a Wiley mill and by exposing the lots of ground tissues to known quantities of HCN in the distillation flask

TABLE 7

AMOUNTS OF HCN RECOVERED FROM GROUND AND FUMIGATED CITRUS LEAF TISSUE AND FROM CITRUS LEAVES THAT HAD BEEN WASHED TO REMOVE HCN ADHERING TO THEIR SURFACES

Material used	Experiment No.	Moisture in leaves, per cent	HCN added, mg	HCN recovered	
				Amount, mg	Per cent
Washed leaves	232	59 5	38 7	26 4	68 2
	233	59 5	43 0	26 4	61.4
	235	59 5	28 8	20 6	71 5
	236	59 4	46 8	29 9	63 9
	Average				66 3
Ground leaf tissue . . .	211	59 7	0 0	0 0	0 0
	207	60 0	42 6	33.7	79 1
	208	61.5	23 8	19 1	80.3
	209	57 6	47 5	36.8	77 5
	210	60 5	27 8	23.1	83.1
	Average				80 0

for a period of 45 minutes before distillation was begun. In these tests, the average recovery of HCN was only 80.0 per cent, as shown in the second group of data in table 7.

Several series of experiments were carried out to determine the effects of different experimental treatments on the amounts of HCN that could be recovered from leaves. The treatments used are shown in table 8. The double-distillation process was used in all of the tests, and either gas or

TABLE 8

AMOUNTS OF HCN RECOVERED FROM 200-GRAM LOTS OF CITRUS LEAVES FUMIGATED AND DISTILLED UNDER DIFFERENT EXPERIMENTAL CONDITIONS

Treatment*	Experiment No.	Moisture in leaves, per cent	HCN added, mg	HCN recovered		HCN not recovered by distillation	
				Amount, mg	Per cent	Amount, mg	Per cent
Series A, 100 grams of leaves, fumigated 45 minutes							
Solutions acidified with H_2SO_4	180	54 3	51 8	47 1	90 9	4 7	9 1
	181	52 5	31 7	28 2	89 0	3 5	11 0
	182	53 5	29 6	26 5	89.5	3 1	10 5
	183	52 2	32 9	29 6	90 0	3 3	10 0
	Average				89 9		10 1
Solutions acidified with tartaric acid.	184	55 3	39 8	35 1	88 2	4 7	11 8
	185	55 5	41 9	37 2	88 8	4 7	11 2
	186	54 8	47 9	41 0	85 6	6 9	14 4
	187	56 0	48 3	43 4	89 9	4 9	10 1
	188	53 8	13 4	10 9	81 3	2 5	18 7
	Average				86 8		13 2
Series B, 200 grams of leaves, fumigated 90 minutes							
Solutions acidified with H_2SO_4	195	57 8	32 8	26 5	80 8	6 3	19 2
	196	58 0	34 8	29.2	83 9	5 6	16 1
	197	59 0	47 5	39 9	84 0	7 6	16 0
	198	60 0	38.4	32 6	84.9	5 8	15 1
	Average				83 4		16 6
Solutions acidified with tartaric acid	189	56 2	28.7	20 4	71 1	8 3	28 9
	190	56 7	31 9	25 1	78.7	6 8	21 3
	191	55 7	42 7	33 5	78 5	9 2	21 5
	192	55 0	48 9	39.1	80 0	9 8	20 0
	193	53 9	25 5	17 7	69 4	7 8	30 6
	Average				75.5		24 5
No acid in first distillation, H_2SO_4 used in second	202	61.5	23.3	18 8	80 7	4 5	19.3
	203	60 7	41 4	36 0	87.0	5 4	13 0
	204	55 1	37 8	33.3	88 1	4 5	11 9
	205	58 3	23 5	19 1	81 3	4 4	18 7
	Average				84.3		15.7

* All distillations were made by using direct gas or electric heat, except as indicated for the second group of tests in series C

TABLE 8 (*Continued*)

Treatment*	Experiment No.	Moisture in leaves, per cent	HCN added, mg	HCN recovered		HCN not recovered by distillation	
				Amount, mg	Per cent	Amount, mg	Per cent
Series C, 200 grams of leaves, fumigated 45 minutes							
No acid in first distillation, H_2SO_4 used in second	194	54.8	40 7	33 2	81 6	7 5	18.4
	199	59 7	33 1	29 0	87 6	4 1	12 4
	200	56 3	53.0	45 7	86 2	7 3	13 8
	201	60 5	46 0	40 1	87 2	5 9	12 8
	Average				85 6		14 4
Steam distillation, solutions acidified with H_2SO_4.	224	59 5	56 8	50 1	88 2	6 7	11 8
	226	61 7	45 8	38 5	84 1	7 3	15 9
	228	58 6	49 4	42 2	85 4	7 2	14 6
	229	57 3	34 9	28 9	82 8	6 0	17 2
	Average				85 1		14 9
Solutions acidified with H_2SO_4	220	59 8	37 3	32 5	87 1	4 8	12 9
	221	57 7	54 7	47 4	86 7	7 3	13 3
	273	62 0	51 3	45 5	88 7	5 8	11 3
	274	62 0	45 0	38 9	86 4	6 1	13 6
	Average				87 2		12 8
Series D, 200 grams of young leaves, fumigated 45 minutes							
Solutions acidified with H_2SO_4	212	76 4	38 7	31 7	81 9	7 0	18 1
	213	75 5	37 6	26 7	71 0	10.9	29 0
	214	75 9	38 6	26 1	67 6	12 5	32 4
	216	73 4	47 6	31 9	67 0	15 7	33 0
	217	75 9	39 6	32 4	81 8	7 2	18 2
	215	74 3	0 0	0 0	0 0	0 0	0 0
	Average				73 9		26.1

* All distillations were made by using direct gas or electric heat, except as indicated for the second group of tests in series C.

electric heat was applied directly to the distillation flask, except as indicated for the second group in series C. All leaves were mature except those in series D, which were about two-thirds mature. The temperature during the fumigation periods was always between 21° and 25° C, and in most cases it was 22° to 23°. The leaves were not removed from the flask before making the distillation, as had been done in the tests recorded in table 5. The distillation solution was run into the flask through the separatory funnel and none of the HCN was allowed to escape. The recoveries therefore represent not only the recoverable portion of the HCN that had been absorbed by the leaves but also that which remained unabsorbed in the flask at the end of the fumigation period.

Series A of table 8 shows that the average percentage of HCN recov-

ered from 100-gram lots of leaves fumigated for 45 minutes and distilled from water acidulated with H_2SO_4 was 89.9 per cent, and those acidulated with tartaric acid gave a recovery of 86.8 per cent.

In table 8, series B, 200-gram instead of 100-gram lots of leaves were used. The treatments were similar except that the fumigation periods were 90 instead of 45 minutes and that in the last four tests in this series the leaves were distilled in nonacidulated water. The average recoveries for the three groups in this series were: acidulated with H_2SO_4, 83.4 per cent; acidulated with tartaric acid, 75.5 per cent; and nonacidulated, 84.3 per cent.

In series C of table 8, the treatment of the first group was the same as that of the last group in series B, except that the fumigation periods were 45 minutes instead of 90. The average percentage of HCN recovered was 85.6. The second and third groups of data in series C show the comparative effects of steam distillation and distillation with direct heat. The average percentages of recovery of HCN were 85.1 and 87.2, respectively. Although no statistical studies have been made, the variations within the groups indicate that the differences are not significant.

A comparison of the amounts of HCN recovered from mature leaves and from those having reached only about two-thirds full size can be made from the results recorded in the last group of series C (mature) in table 8, and in series D (young). The average percentages of HCN recovered were 87.2 and 73.9, respectively.

THE RECOVERY OF HCN FROM CITRUS-FRUIT DISTILLATES

The green and mature citrus fruits tested were all picked from the same block of trees, a standard orange clipper being used to cut the stem. After picking they were immediately brought to the laboratory, counted into lots—usually of 15 each—weighed, and the diameters measured.

In working with the young, green orange fruits, the same precautions as had been used with the leaves were necessary. In the first tests, the green fruits were cut into small pieces and treated like the leaves. A known quantity of HCN was dissolved in a solution of NaOH of the usual strength, placed in the flask with the sliced fruits, and distilled at once.

In addition to H_2S, which had caused trouble in making determinations from the leaf extracts and which was taken care of by using $CdSO_4$, there appeared to be a substance or substances in the distillate which slowly reacted with the HCN. In order to check on this possibility, the amounts of HCN in the distillates were determined immediately after distillation and then again after the distillate had stood for 24 hours.

The results of these tests are shown in table 9. The quantities of HCN added before distillation plotted against the quantities recovered in the distillate immediately after distillation show a straight-line relation (graph not included in this paper).

TABLE 9

AMOUNTS OF HCN RECOVERED FROM THE DISTILLATE FROM GREEN AND MATURE VALENCIA FRUITS

(The HCN was added either immediately before or after distillation; there was no fumigation period)

HCN added before or after distillation	Experiment No.	Date of picking, 1937–38	Weight of fruits,* grams	HCN added, mg	HCN recovered after standing: Amount, mg, 0 hours	HCN recovered after standing: Amount, mg, 24 hours	HCN recovered after standing: Per cent, 0 hours	HCN recovered after standing: Per cent, 24 hours
Green Valencia fruits								
Before	287	Oct 20	729	52.8	48.4	.	91.7	...
	288	Oct. 20	724	37.2	34.4		92.5	
	289	Oct. 25	1,103	39.6	36.4		91.9	..
	290	Oct. 25	1,208	48.6	44.6	.	91.8	
	339	Nov. 26	1,140	81.6	73.5	71.9	90.1	88.1
	340†	Nov. 26	1,108	27.1	25.6	24.7†	94.5	91.1†
	360	Dec 17	1,144	65.5	60.7	59.6	92.7	91.0
	361	Dec. 17	1,242	42.3	39.4	37.7	93.1	89.1
	362	Dec. 20	1,341	16.7	15.2	14.3	91.0	85.6
	363	Dec. 20	1,300	23.9	22.5	21.6	94.1	90.4
	364	Dec 21	1,464	34.6	31.7	30.1	91.6	87.0
	365	Dec 21	1,390	28.5	27.2	25.6	95.4	89.8
	Average						92.5	89.0
Immediately after	342‡	Nov 29	2,248	75.0	73.1	69.6‡	97.5	92.8‡
24 hours after	343	Dec. 1	1,155	15.1	14.5	14.3	96.0	94.7
48 hours after	344	Dec. 1	1,117	39.2	37.8	37.4	96.4	95.4
Mature Valencia fruits								
Before	366	May 3	1,182	71.6	67.4		94.1	.
	367	May 4	1,085	34.2	31.4	30.6	91.8	89.5
	368	May 4	1,122	35.6	33.0	32.2	92.7	90.4
	369	May 5	1,184	50.0	45.8		91.6	
	Average						92.5	90.0

* The number of fruits was 15 except as follows: experiment No. 287, 13 fruits; No. 288, 13; No. 342, 30; No. 366, 7; No. 367, 7; No. 368, 8; and No. 369, 8

† After 72 hours, 23.7 mg, 87.5 per cent

‡ After 48 and 72 hours, 69.0 and 68.5 mg, 92.0 and 91.3 per cent, respectively.

Further trials were made, not only by adding a known quantity of HCN immediately before distillation and by making determinations on the distillate at intervals, but also by adding known quantities of HCN to distillates from unfumigated fruits and by making determinations at once and at 24- and 48-hour intervals. The results of these tests are also

given in table 9. The leaf extracts appeared to contain similar substances, but no definite tests were made to determine the amounts of HCN that combined with them after given intervals of time.

Several months later similar tests were made on mature Valencia fruits taken from the same trees. The color of the distillate indicated that the amount of H_2S in the mature fruits was less than that in the green fruits; however, enough was present so that it was necessary to use $CdSO_4$ in the distillation flask. The amounts of HCN recovered from the different lots of mature fruits are given in the lower section of table 9.

In the next series of tests, different lots of green fruits were fumigated for 40 minutes in a 100-cu. ft. gastight metal fumatorium with different amounts of HCN.

The amounts of HCN used for fumigation at different times ranged from 0.88 mg to 3.66 mg per liter of space in the fumatorium. The fumigation temperature was always maintained at 24° C. The period of fruit aeration after fumigation ranged from 5 minutes to 44 hours; the two periods most used were 10 minutes and 22 hours. A fan kept the gases in the fumatorium in constant circulation during the entire fumigation period. The concentration of HCN in the fumatorium was measured four times during a fumigation period.

After fumigation, each fumigated fruit was cut into 10 to 15 pieces and placed in the distillation flask with 3 liters of acidulated distilled water and 30 grams of $CdSO_4$. It was necessary to make only a single distillation with the fruits because the tissues were more nearly completely immersed in the distillation liquid. In experiments 281 to 334, inclusive, the fruits were distilled by direct heating. In experiments 337 to 369, inclusive (Nos. 342 to 369 not included in table 10), they were steam-distilled. One liter of distillate was caught in NaOH as described for the leaves. After filtering the distillate, 150-ml aliquots were used for titration.

Table 10 shows the amounts of HCN recovered from green Valencia oranges that had been fumigated in the fumatorium. Four to eight lots of fruit were used in each experiment. The amount of HCN recovered was roughly in direct proportion to the concentration of HCN in the fumatorium, as shown in figure 1. The figure does not show it, but the amount of HCN recovered from the fruit was inversely proportional to the length of the aeration period. No HCN could be detected after 44 hours' aeration.

The data recorded in table 11 are similar to those in table 10, except that, although in all cases the whole fruits were fumigated, the peels and pulps of some lots were distilled separately. This was done in order to

TABLE 10

Amounts of HCN Recovered from the Distillate from Green Valencia Oranges Fumigated with Different Amounts of HCN in the Fumatorium

HCN per liter in fumatorium, mg	Date of picking fruits, 1937	Experiment No.	Length of aeration	Weight of fruits, grams	Average diameter of fruits, inches	HCN recovered, mg	
						Sample of 15 fruits	Average of 2 samples
		281	5 min.	1,120	$2\frac{3}{32}$	8.8	8.3
		282	5 min.	1,201	$2\frac{1}{8}$	7.7	
1.10	Oct. 20	283	22 hrs.	1,254	$2\frac{5}{32}$	1.0	0.8
		284	22 hrs.	1,037	2	0.6	
		285	44 hrs.	1,063	2	0.0	0.0
		286	44 hrs.	1,211	$2\frac{1}{8}$	0.0	
		323	15 min.	1,171	$2\frac{1}{8}$	12.1	12.5
0.88	Nov. 17	324	15 min.	1,203	$2\frac{1}{16}$	13.0	
		325*	22 hrs.	1,174	$2\frac{1}{8}$	2.1	2.0
		326*	22 hrs.	1,182	$2\frac{1}{8}$	1.9	
1.61	Oct. 29	299	15 min.	1,206	$2\frac{1}{8}$	21.4	22.1
		300	15 min.	1,142	$2\frac{1}{16}$	22.9	
1.52	Nov. 3	305	10 min.	1,111	$2\frac{1}{16}$	27.7	29.3
		306	10 min.	1,150	$2\frac{1}{16}$	31.0	
		311	10 min.	1,175	$2\frac{1}{8}$	21.0	19.6
1.47	Nov. 9	312	10 min.	1,142	$2\frac{1}{16}$	18.3	
		315	24 hrs.	1,184	$2\frac{1}{8}$	2.6	2.6
		316	24 hrs.	1,123	$2\frac{1}{16}$	2.6	
		291	5 min.	1,174	$2\frac{1}{8}$	38.6	39.0
2.58	Oct. 26	292	5 min.	1,191	$2\frac{4}{32}$	39.3	
		295	44 hrs.	1,164	$2\frac{3}{32}$	0.0	0.0
		296	44 hrs.	1,190	$2\frac{4}{32}$	0.0	
2.58	Oct. 27	293	22 hrs.	1,160	$2\frac{3}{32}$	4.7	5.1
		294	22 hrs.	1,148	2 3/[illegible]	5.6	
2.46	Nov. 12	317	10 min.	1,051	$2\frac{1}{16}$	35.2	35.6
		318	10 min.	1,106	$2\frac{1}{8}$	36.1	
3.66	Nov. 15	319	10 min.	1,140	$2\frac{1}{8}$	68.4	66.6
		320	10 min.	1,085	$2\frac{1}{16}$	64.7	
3.15	Nov. 16	321	10 min.	1,120	$2\frac{1}{16}$	58.2	59.0
		322	10 min.	1,022	$2\frac{1}{16}$	59.8	
		327*	15 min.	1,126	$2\frac{1}{16}$	57.3	54.0
3.21	Nov. 19	328*	15 min.	1,129	$2\frac{1}{16}$	50.8	
		329	22 hrs.	1,143	$2\frac{1}{16}$	8.8	9.1
		330	22 hrs.	1,170	$2\frac{1}{8}$	9.4	
		331‡	15 min.	1,146	$2\frac{1}{8}$	39.9	43.3
3.24	Nov. 22	332‡	15 min.	1,134	$2\frac{1}{8}$	46.6	
		333†	22 hrs.	1,136	$2\frac{1}{8}$	6.4	6.7
		334†	22 hrs.	1,155	$2\frac{1}{8}$	6.9	
		337	15 min.	1,065	$2\frac{1}{16}$	32.8	32.2
2.50	Nov. 26	338	15 min.	1,075	$2\frac{1}{16}$	31.7	
		341	22 hrs.	1,177	$2\frac{1}{8}$	6.6	

*, ‡, † Tested 24, 42, and 72 hours, respectively, after distillation. No earlier titrations were made.

determine the depth of penetration of the HCN into the fruits during the fumigation period and during the interval between fumigation and distillation. In order to guard as much as possible against the loss of HCN, the fruits were dipped in distilled water before peeling and the water was added to the distillation solution. In this connection it may be

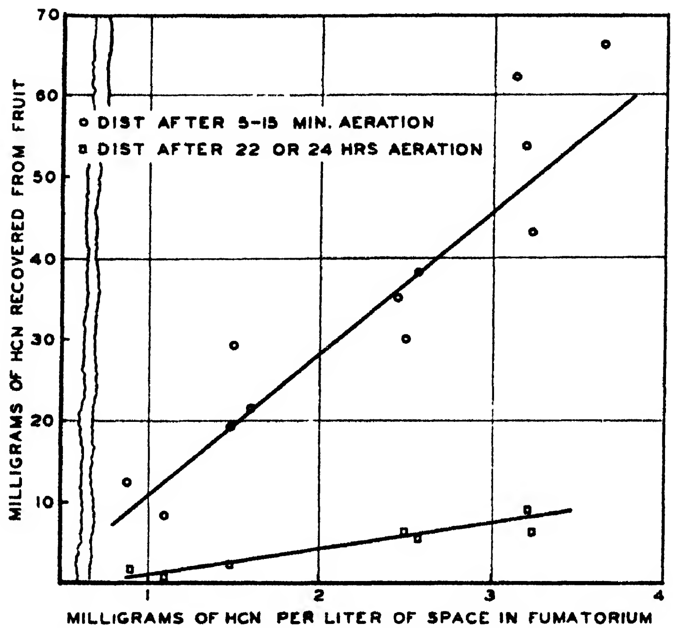

Fig. 1.—The amounts of HCN that were placed in the 100-cu.-ft. fumatorium with different lots of green Valencia fruits, and the amounts of HCN recovered from them by subsequent distillations (see table 10).

mentioned that citrus fruits are acid and such a condition favors the release of HCN. The determinations were made on all of the distillates immediately, and then again 24 or 48 hours later.

DISCUSSION

A nonvolatile organic acid of low concentration is usually recommended and used in the recovery of HCN from biological materials by the distillation method. Krieble and Peiker (*5*) found that hydrolysis of the HCN will occur, and Cobb and Walton (*4*) have shown that HCN forms complexes with H_2SO_4, if the acid concentrations are too high. However, Pagel and Carlson (*7*) and Morris and Lilly (*6*) reported satisfactory recovery when HCN was distilled from solutions acidified with H_2SO_4.

Most of the distillations reported in these experiments were made in the presence of H_2SO_4. This acid was used because, as shown in series A

and the first two groups of series B in table 8 (p. 482), a greater percentage of the HCN could be recovered by using H_2SO_4 than by using tartaric acid. Furthermore, for some unknown reason, titration to the end point was somewhat more difficult when tartaric acid was used. As has been stated already, in the earlier tests, 0.30 ml, but in the later tests only 0.15 ml, of concentrated H_2SO_4, beyond neutrality, was used in the 3 liters of solution in the distillation flask. It should be borne in mind

TABLE 11

AMOUNTS OF HCN RECOVERED FROM FUMIGATED GREEN VALENCIA ORANGES AND FROM THEIR PEELS AND PULPS SEPARATELY

(The determinations were made immediately after distillation and 24 or 48 hours later)

HCN per liter in fumatorium, mg	Date of picking, 1937	Length of aeration	Average diameter of fruits, inches	Weight of fruits, grams	Experiment No.	Part of fruit distilled	HCN recovered after standing, mg			HCN lost on standing, mg	
							0 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.
2.50	Nov. 26	15 min.	2 1/16	1,065	337	Whole	32.8	31.7		1.1	
		15 min	2 1/16	1,075	338	Whole	31.7	29.7		2.0	
		22 hrs.	2 1/8	1,177	341	Whole	6.6		5.0		1.6
1.68	Nov. 24	15 min	2 1/16	1,087	335	Peel	19.8		17.7		2.1
					336	Pulp	2.6		2.0		0.6
1.59	Dec. 2	15 min.	2 1/16	1,149	345	Peel	15.8	14.4		1.4	
					346	Pulp	3.2	2.3		0.9	
		4 hrs	2 1/8	1,213	347	Peel	5.5	5.0		0.5	
					348	Pulp	1.9	1.2		0.7	
		22 hrs	2 1/16	1,133	349	Peel	2.0	1.9		0.1	
					350	Pulp	0.6	0.4		0.2	

that neither of these concentrations of H_2SO_4 (0.0176 per cent, 0.0036 *N*, and 0.0088 per cent, 0.0018 *N*, respectively) furnished the solutions with as many free H-ions as were available in the 2 per cent (0.2666 *N*) tartaric acid solution. Table 4 (p 479) shows that the same average recovery of HCN was obtained with H_2SO_4 as with tartaric acid when the distillations were made in the absence of leaves.

Distillations were made with steam or, more often, with heat from a free flame or an electric heater. The results given in treatment C of table 2 (p. 475) show that the application of direct heat did not cause a measurable destruction of HCN, probably because of the high vapor pressure of HCN, which caused it to distill rapidly, and because of its very low concentration in the solution in the distillation flask.

The liquid HCN used in the experiments reported in this paper was

guaranteed to contain 96 per cent available HCN. Corrections have accordingly been made to a basis of 100 per cent available HCN wherever percentages of recovery are given. However, when the distillations were made in the absence of tissues, the recoveries (see table 2, treatment B, and table 4) indicate that the amount of available HCN was approximately 96.5 per cent.

All of the HCN could not be recovered from citrus leaf tissues. Repeated tests showed that no additional HCN could be recovered from the fumigated tissues by driving over more than 800 to 900 ml of distillate or by redistilling the tissues. Tests also showed that none of the HCN was escaping from the receiving vessel. This was determined by using a chain of receiving vessels. All of the HCN was caught in the first unit.

The leaves appear to contain a substance or substances which combine with a portion of the HCN in such a manner that it either is no longer volatile, or if it does distill over, will not react with standard $AgNO_3$. More HCN could be recovered when the distillations were made as soon as the HCN had been added than when the tissues were exposed to it during a 45-minute fumigation period. This is shown very well by the data in tables 6 and 8. Table 6 (p. 481) shows that 99.0 per cent of the HCN could be recovered when it was distilled at once from leaf distillate and 95.8 per cent when distilled at once in the presence of unfumigated leaves. These percentages of recovery are considerably higher than those obtained by making the distillations after a 45-minute fumigation period. Under such conditions the highest percentages of recovery from 100-gram and 200-gram samples of mature leaves were 89.9 per cent and 87.2 per cent, respectively, while the recovery from 200-gram samples of immature leaves was only 73.9 per cent (see table 8, p. 482). Such results indicate that at least a certain amount of HCN can be chemically bound or fixed by citrus leaf tissues. Apparently biological or other factors prevented proportionality fixation.

Because of the manner in which the fruits were fumigated, the percentages of HCN recovered from them cannot be computed. That the fruits, as well as the leaves, fixed a certain amount of the HCN during the fumigation period is indicated by the fact that when unfumigated fruits were placed in an alkaline HCN solution and distilled at once, only about 92 per cent of the HCN could be recovered (table 9, p. 485). This is more strikingly shown in results that have not been described or tabulated. When different lots of fruits were exposed to 1.61 mg of HCN per liter of space in the fumatorium and distilled, after a 15-minute period of aeration, about 22 mg of HCN was recovered. However, when other lots of fruit, which had been fumigated at the same time, were placed in

desiccators over NaOH and left for 17 and 64 hours, averages of only 8.9 mg and 2.3 mg, respectively, could be recovered from the fruits and their underlying NaOH.

In studying the cyanophoric glucosides in such plants as *Prunus virginiana* and *Andropogon Sorghum*, Alsberg and Black (*1*) found that not only all autogenous HCN in the tissues could be obtained but also that 100 per cent of any added HCN could be recovered if the distillation were made at once. These results do not agree with those reported in this paper for citrus tissues. Immediate distillation did not recover all of the added HCN, and the more finely divided the tissues were before being fumigated or the younger the tissue, the less the percentage that could be recovered. Only about 80 per cent could be recovered when the mature leaf tissues were finely ground, fumigated, and distilled at once (table 7, p. 481), and only about 74 per cent from whole, immature leaves (table 8, p. 482). From these and other studies (*10*) it appears that the amount of HCN fixed or chemically changed during and after fumigation depends upon the kind of plant tissue that is being tested.

In this connection it may be repeated that Bartholomew and Raby (*3*) found that citrus leaf and fruit tissues do not contain a cyanophoric glucoside. These results have been verified in the present investigation (for example, see experiment 211, table 7, p. 481).

Although studies in this field have been outlined, no attempt was made during the course of these experiments to determine the nature or identity of the substances which prevented the recovery of all of the HCN. Bartholomew and Raby (*3*) found that full recoveries could be made when HCN was distilled in the presence of sugars, citral, or pure citrus oils. They found, however, that small amounts of aldehydes can be recovered from green citrus tissues and that these may be at least partially responsible for the fixation of the HCN that could not be recovered after distillation in the presence of the tissues. Whether these substances or others were responsible for the progressive disappearance of titratable HCN from the distillate from citrus tissues upon standing is not known. Maximum determinations of HCN in the distillates were obtained by titrating immediately after distillation.

That the disappearance of HCN, in such dilute solutions as were used, could not have been caused except by its being combined with substances in or distilled from the tissues was determined by experimentation. Thirty-one mg of NaCN were dissolved in 1 liter of distilled H_2O, and 125 liters of air per hour were bubbled through it over a period of 24 hours. At the end of the period the solution contained the same amount of NaCN as at the beginning.

That the HCN had actually penetrated the leaves during the period of fumigation and was not merely adhering to their surfaces was shown by the fact that an average of 66.3 per cent of the HCN could be recovered from the leaves even after they had received the several washings of alkaline, distilled, and acidulated water (see table 7, p. 481). Similar results are indicated in table 5 (p. 480), where it is shown that HCN could be recovered from mature citrus leaves although they had been exposed to the laboratory air for as long as 15 hours. By the end of the 15-hour period, the leaves had become badly wilted and had lost about 27 per cent of their water content. Further evidence that the HCN penetrates into the citrus tissues is presented in table 11 (p. 489), which shows that HCN could be recovered from the pulps after the peels had been removed from fumigated whole fruits. These results are specially mentioned because of unpublished statements to the effect that HCN does not enter citrus leaves or fruits but merely adheres to their cutinized surface during the fumigation period of 40 or 45 minutes.

The first group of results given in table 5 (p. 480) were obtained by removing the leaves from the fumigation flask, exposing them to the laboratory air, and stirring them for 1 minute before distilling them to recover the HCN. The question may be raised as to whether all of the HCN had escaped from the surface of the leaves during such a short period of time. That such a time interval was sufficient is indicated by the fact that the average of the amounts of HCN recovered from leaves treated in this manner was 66.8 per cent (table 5), while the average recovered from the leaves that had received the several washings, after being fumigated and before being distilled, was 66.3 per cent (table 7, p. 481).

The results given in tables 10 and 11 (pp. 487 and 489) are expressed in milligrams rather than as percentages. Recovery percentages, calculated on the basis of the total quantity of HCN added to the fumatorium and the amount recovered from the fruit would be of little value, since a considerable quantity of the HCN was sorbed on the walls of the fumatorium; HCN is known to be strongly sorbed on surfaces of this kind. Furthermore, traces of sodium or other alkaline substances on the walls of the fumatorium would also react with the HCN. Early investigations showed that soda glass could not be used in such experiments.

As has been stated, tests were made, usually at intervals of 2, 7, 15, and 30 minutes, in order to determine the relative concentrations of HCN to which the tissues were exposed in the fumatorium during the fumigation period. The maximum decrease in the amount of HCN in the fumatorium at the time of the 30-minute test, in comparison with that present at the

time of the 2-minute test, was 14.4 per cent. The average decrease for all fumatorium tests was 8.2 per cent.

Results of unpublished experiments[7] show that this average of 8.2 per cent is very close to the average obtained when tests were made to determine the amounts of HCN that would be sorbed by the walls, pipes, and other portions of the iron fumatorium when no tissues were present. Therefore only a comparatively small proportion of the decrease in HCN in the fumatorium by the end of the fumigation period was due to absorption by the fruit that was being fumigated. However, computations from the data in table 10 (p. 487) indicate that a given volume of fruit contained from ten to fourteen times as much HCN as an equal volume of air in the fumatorium. The amounts of HCN to which the different lots of fruits were exposed, as given in tables 10 and 11 (pp. 487 and 489), are the averages of the tests made during the fumigation period and not the original amounts placed in the fumatorium.

In a preliminary report, Bartholomew and Raby (*3*) stated that a comparatively large amount of HCN was combined or fixed by substances distilled from citrus leaves in such a manner that it would not react with standard $AgNO_3$. The present investigations have shown that the amounts of HCN fixed by citrus tissues (table 6, p. 481) are not so great as they stated. The reason for the error in the preliminary work was the presence of H_2S in the distillate, which prevented the determination of the end point until excessive amounts of HCN had been added. The placing of $CdSO_4$ in the distillation flask obviated the difficulty encountered in the earlier work because it changed the volatile H_2S to nonvolatile CdS.

SUMMARY

Methods are described for the handling of HCN in amounts as small as 10 to 15 mg and for the distillation, recovery, and determination of HCN from citrus tissues.

Hydrogen sulfide from the fumigated citrus leaves and fruits passed over into the distillate and interfered with the determinations of HCN with standard $AgNO_3$. This trouble was overcome by placing $CdSO_4$ in the distillation flask at the time of making the first (fruits) or second (leaves) distillation.

Tissues of citrus leaves and fruits are already acid, so that only a small amount of concentrated H_2SO_4 had to be added to insure the recovery of the HCN during distillation. No destruction of the HCN occurred as a

[7] Lindgren, D. L. Sorption of HCN by the walls of a metal fumatorium. Unpublished material on file at University of California Citrus Experiment Station, Division of Entomology. 1935.

result of using H_2SO_4 of these concentrations, and even better recoveries of HCN were obtained than when the tissues were distilled in the presence of a 2 per cent solution (beyond neutrality) of tartaric acid.

The experimental results indicate that citrus leaves and fruits fix or alter a portion of the HCN during the fumigation period so that it cannot be recovered by distillation. Approximately 85 per cent of the HCN could be recovered from mature leaves and 73 per cent from immature leaves. Less HCN could be recovered from leaves that had been finely ground before being fumigated than from fumigated whole leaves. There was an evident fixation of a portion of the HCN. HCN penetrates into the tissues and does not merely adhere to the surface. This was shown by the tests in which the leaves were thoroughly washed before distillation and by the fact that HCN could be recovered from the pulp of fumigated fruits after they had been peeled.

Aeration tests with mature fumigated leaves showed that the amounts of HCN that could be recovered from them decreased in roughly inverse proportion to the length of time of aeration. Fifteen per cent was recovered from the leaves after 15 hours, but none could be recovered from the fruits after 44 hours.

The amounts of HCN that could be recovered from citrus leaves and fruits were directly proportional to the amounts of HCN placed in the fumatorium in these tests.

When leaf and fruit distillates which contained HCN were allowed to stand, some unknown substance in the distillate continued to combine slowly with the HCN so that it would not react when titrated with standard $AgNO_3$.

By the end of the 40-minute fumigation period in the fumatorium, a given volume of the green fruit contained from ten to fourteen times as much HCN as there was in an equal volume of air in the fumatorium.

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Sincere acknowledgments are made to the Division of Entomology of the Citrus Experiment Station for the use of its very efficient experimental fumatorium, and especially to Dr. David Lindgren of that division, who fumigated the different lots of fruit and made the four concentration tests on the HCN in the fumatorium at each fumigation.

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CONTENTS

③# H I L G A R D I A

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FACTORS AFFECTING CURLY-TOP INFECTIVITY OF BEET LEAFHOPPER, EUTETTIX TENELLUS[1]

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INTRODUCTION

A NUMBER OF INVESTIGATORS have called attention to the fact that large numbers of beet leafhoppers, *Eutettix tenellus* (Baker), collected in the foothill breeding areas and on weeds in the cultivated areas, failed to transmit the curly-top virus to sugar beets. There have been occasional reports in the literature of large populations of leafhoppers in beet fields with a small amount of curly top developing during the season.

Smith and Boncquet (*23*)[3] tested fully 2,000 beet leafhoppers taken on *Atriplex tularensis* and *Chenopodium album* in the Tulare Lake region of the San Joaquin Valley upon several hundred sugar-beet plants without the production of curly top in a single instance.

Boncquet and Hartung (*1*) report that 100 leafhoppers collected on species of *Artemisia* and *Atriplex* in the Tulare Lake region and confined singly in cages on beet seedlings failed to produce curly top. Hartung (*5*), in a detailed paper on the results of the same experiment, states that 87 insects were tested and that 7 per cent "probable" cases of curly top developed. These "probable" cases of curly top showed one or more slightly curled leaves, but no reliable symptoms of the disease, such as wartlike protuberances on the lower surface of the leaves.

Hartung (*6*) tested 2 lots of leafhoppers collected on the foothills, and

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[3] Italic numbers in parenthesis refer to "Literature Cited" at the end of this paper.

found that 6 per cent of one and 30 per cent of the other transmitted the curly-top virus to sugar beets.

Hartung (*5*) found the beet leafhopper abundant in a field of beets just being thinned at Visalia on April 20, 1915, and when he again visited the beet field on June 5, only slight indications of curly top were present. He concluded that a high percentage were unable to transmit the curly-top virus.

A number of lines of investigation were followed in an attempt to determine the percentage of leafhoppers infective in different localities in the state and in different years, and to analyze the factors that may be involved. To this end, tests were made to determine whether leafhoppers collected many miles from sugar-beet fields are infective; what percentage of leafhoppers are infective on the foothills of the San Joaquin and Salinas valleys and in the cultivated areas; whether food plants immune to curly top affect the period of infectivity of the insect; what percentage of infectivity is shown by insects reared on weeds that attenuate the curly-top virus; whether the virus can be recovered from infected perennials repeatedly and at different seasons, and from infected annuals repeatedly during the life of the plants. Some further tests were also conducted on the host range and reservoirs of curly top on the uncultivated plains and foothills, and on the recovery of the virus from the most important breeding plants by single insects.

METHODS

In collecting beet leafhoppers under natural conditions, general sweepings were made on pasture vegetation, preferably on one species of plant, although this was not always possible. The leafhoppers taken on each species of plant were removed from the insect net with a pipette described in a previous paper (*15*) and transferred to a cage. On red-stem filaree (*Erodium cicutarium*) growing on the foothills, nymphs were usually captured with a pipette. In beet fields the overwintering females were disturbed by moving the hand among the leaves, and then the insects were caught with a pipette. The vegetation on which leafhoppers were captured was not used as food because there was a possibility that some of the plants were infected with curly top, and hence the insects while in the field were fed in cages on the foliage of Mammoth or Alameda sweet corn (*Zea Mays*), which is immune to curly top.

In determining the percentage of infective beet leafhoppers under natural conditions, from 25 to 100 nymphs or adults were collected on the foothills or in the cultivated areas, and then each specimen was confined in a cage enclosing a healthy sugar-beet seedling in the greenhouse.

During the years 1926 to 1933 but not during 1924, each leafhopper was fed on the first beet for a period of 2 weeks and then was provided with a second healthy beet, except those specimens which had transmitted the curly-top virus to the first plant.

INFECTIVITY OF BEET LEAFHOPPERS IN DESERT, FOOTHILL, AND CULTIVATED AREAS

Near and Away from Beet Fields.—In 1918, tests were made to determine whether beet leafhoppers were infective in localities where no sugar beets were grown and also in the vicinity of beet fields. The results are given in table 1. Adults were collected on different species of plants

TABLE 1

PLANTS FROM WHICH BEET LEAFHOPPERS TRANSMITTED CURLY-TOP VIRUS TO SUGAR BEETS

District	Plants on which insects were collected		Number of insects tested	Date insects were collected, 1918
	Common name	Scientific name		
Imperial Valley, away from beet fields:				
Dixieland	Lowland purslane	*Sesuvium sessile*	7 adults	Mar. 13
Calexico	Lowland purslane	*Sesuvium sessile*	300 adults	Apr. 2
Niland	Wheelscale	*Atriplex elegans*	25 nymphs	Apr. 21
			200 adults	Apr. 21
Mojave Desert, 2 to 4 miles from beet fields near Victorville	Creosote bush	*Larrea tridentata* var. *glutinosa*	14 adults	Jan. 30
Salinas Valley:				
King City, near beet fields	Red-stem filaree	*Erodium cicutarium*	3 adults	May 27
King City, foothills	Red-stem filaree	*Erodium cicutarium*	3 nymphs	Nov. 28
Bitterwater, foothills	Red-stem filaree	*Erodium cicutarium*	100 adults	Oct. 13
San Joaquin Valley:				
Foothills 13 miles southwest of Tracy	Red-stem filaree	*Erodium cicutarium*	12 adults	Dec 10
			18 nymphs	Dec. 24
			25 adults	Dec. 24

growing in the Imperial Valley, where no sugar beets were grown during 1918, and each lot, varying in number from 7 to 300 specimens, transmitted the curly-top virus to sugar beets. In the Mojave Desert, leafhoppers collected on creosote bush (*Larrea tridentata* var. *glutinosa*), from 2 to 4 miles away from beet fields near Victorville, were found to be infective; the adults were collected on January 30 after the beets had been harvested. Nymphs and adults collected on the foothills of the Salinas and San Joaquin valleys transmitted the curly-top virus to sugar beets. Beets were grown near King City in the Salinas Valley and near

Tracy in the San Joaquin Valley, but not near Bitterwater, situated in a mountain pass in the Gabilan Mountains.

In Little and Big Panoche Passes.—During 1924, the adults of the spring generation were captured in many localities on the foothills and floor of Little Panoche Pass, situated in the middle San Joaquin Valley. In the greenhouse, 50 males and 50 females were confined singly in 100 cages, each enclosing a healthy beet seedling, and every insect that had not transmitted the virus was transferred every two weeks to a healthy beet seedling for a period of 16 weeks. The experiment was begun on April 10 and ended on August 1.

Nine males and 8 females, or 17 per cent (table 2), transmitted the curly-top virus to sugar beets. Eight males and 7 females infected the first beet. One insect infected the second beet but not the first; another infected the third but not the first two: in other words, periods of at least 14 and 28 days, respectively, elapsed before infections occurred.

In a previous paper (*11*), it was reported that when infective males that had completed the nymphal stages on diseased beets, were provided with a healthy beet daily during adult life, there were periods of from 8 to 56 days in which no infections occurred. The 14- and 28-day periods mentioned in the preceding paragraph may correspond to intervals between infections or may represent long incubation periods of the virus in the beet leafhoppers which had fed for short periods on diseased beets as reported by Freitag (*4*).

During 1926 the percentage of females infective in the overwintering generation was compared with that of the adults infective in the spring generation. In one test, the overwintering females and the adults of the spring generation were collected on *Erodium cicutarium* in many favorable localities in Little Panoche Pass, and in another test the adults of both generations were taken abundantly in the corresponding localities on a foothill near the entrance of Big Panoche Pass. The results are given in table 2. The percentage of infectivity is higher with adults of the spring generation than with the overwintering females. The overwintering adults, however, were collected on March 2, and it has been demonstrated that leafhoppers near the end of their natural life do not produce curly top so often as during early adult life (*4, 11*). The overwintering females die during March and early April on the uncultivated plains and foothills (*15*).

During 1929, 1930, and 1931, only adults of the spring generation were collected and tested, and only in Little Panoche Pass. The results are shown in table 2.

During 1932, the percentages of females infective in the overwinter-

TABLE 2

PERCENTAGE OF INFECTIVE BEET LEAFHOPPERS UNDER NATURAL CONDITIONS

Year and locality	Food plant	Date	Generation	Number	Per cent of infective insects
		Uncultivated plains and foothills, San Joaquin Valley			
1924					
Little Panoche Pass	Pasture vegetation	Apr. 8	Spring	100	17
1926					
Little Panoche Pass	*Erodium cicutarium*	Mar. 2	Overwintering	100	24
		Apr. 12	Spring	100	36
Big Panoche Pass	*Erodium cicutarium*	Mar. 2	Overwintering	100	22
		Apr. 13	Spring	100	56
1929					
Little Panoche Pass	Pasture vegetation	Apr. 10	Spring	100	16
1930					
Little Panoche Pass	*Erodium cicutarium*	Apr. 3	Spring	100	2
1931					
Little Panoche Pass	*Frankenia grandifolia*	Apr. 8	Spring	100	6
1932					
Little Panoche Pass	*Erodium cicutarium*	Mar. 4	Overwintering	100	25
		Mar. 24	Spring	100	31
		Apr. 6	Spring	100	42
Hospital Canyon	*Erodium cicutarium*	Mar. 30	Spring	50	66
1933					
Little Panoche Pass	*Frankenia grandifolia*	Apr. 10	Spring	100	16
Little Panoche Pass	*Erodium cicutarium*	Apr. 11	Spring	100	37
Hospital Canyon	*Erodium cicutarium*	Apr. 18	Spring	100	53
Corral Hollow	*Erodium cicutarium*	Apr. 18	Spring	100	46
Lone Tree Canyon	Pasture vegetation	Apr. 20	Spring	100	15
				100	12
		Uncultivated foothills, Salinas Valley			
1926					
Foothills near San Ardo	*Erodium cicutarium*	Feb. 23	Overwintering	100	65
		Mar. 21	Spring, nymphs	50	100
		Apr. 20	Spring, nymphs	50	100
		Apr. 20	Spring, adults	100	88
1932					
Pine Valley	*Erodium cicutarium*	Mar. 8	Overwintering	100	22
		Apr. 15	Spring	100	17
1933					
Hog Canyon	*Erodium cicutarium*	Apr. 13	Spring	100	16
Foothills near Metz	*Erodium cicutarium*	Apr. 13	Spring	100	9
		Cultivated areas, Salinas Valley			
1926					
Gonzales to King City	Sugar beets	Feb. 24	Overwintering	100	42
King City	Weeds	Apr. 20	Spring	100	36
1932					
King City	Sugar beets	Mar. 6	Overwintering	25	40
Metz	Sugar beets	Mar. 8	Overwintering	100	11
King City	Sugar beets	Apr. 15	Spring	25	12
		Cultivated areas, San Juan Valley			
1932					
San Juan Valley	Sugar beets	Mar. 6	Overwintering	100	43

ing generation was again compared with that of adults infective in the spring generation collected in many localities on different dates in Little Panoche Pass. The infectivity tests are reported in table 2. Here again, a higher percentage of infectivity was obtained with the adults of the spring generation than with the overwintering females. The infectivity of the adults of the spring generation increased from 31 per cent on March 4, to 42 per cent on April 6.

During 1933, adults of the spring generation were collected on two species of food plants in Little Panoche Pass, and tests (table 2) showed more than twice as high a percentage infective on *Erodium cicutarium* as on *Frankenia grandifolia*. *F. grandifolia* is immune to curly top, and leafhoppers would not obtain the virus by feeding on this perennial plant, but, as shown later (p. 514), the period of infectivity in the leafhoppers is probably not affected by the juices from this plant. The period of infectivity of the leafhopper is not shortened by feeding infective adults on Mammoth or Alameda sweet corn (*Zea Mays*), also immune to curly top, and on Australian saltbush, or fleshscale (*Atriplex semibaccata*), which is highly resistant or immune to the disease as discussed later (p. 511).

In Canyons of the Northern San Joaquin Valley.—It has been assumed that higher percentages of infective leafhoppers occur in the northern canyons than in Little and Big Panoche passes of the San Joaquin Valley, owing to the fact that many overwintering adults reared on curly-top beets fly into the northern canyons and spread the virus to susceptible plants. The highest percentage of infectivity of the beet leafhopper in the San Joaquin Valley was obtained in 1932, when 66 per cent of the adults of the spring generation collected in the northern Hospital Canyon transmitted the curly-top virus to sugar beets, as compared with 31 and 42 per cent in Little Panoche Pass.

In 1933 a comparison was made of the percentages of infective adults of the spring generation collected in canyons—Hospital Canyon, Corral Hollow, and Lone Tree Canyon—situated in the northern San Joaquin Valley. The results are given in table 2.

During the spring, beet leafhoppers frequently congregate near the entrance of mountain passes (*16, 18*) and canyons before the flights into the cultivated areas occur. One hundred adults were collected on April 20, 1933, in an area of about 150 square feet of pasture vegetation near the entrance of Lone Tree Canyon, of which 12 per cent proved infective; and another 100 adults were captured in many localities in the same canyon, of which 15 per cent proved infective; the difference is probably not significant.

If the higher percentages of infective adults of the spring generation are associated with the assumption that the overwintering females reared on curly-top beets spread the virus to susceptible plants in the northern canyons, then it would be difficult to explain the lower percentages of infectivity obtained with the adults collected in Lone Tree Canyon, situated only a few miles away from Hospital Canyon. This discrepancy, however, forms a single exception to the general trend and should not be given too much emphasis. Beets were grown near Manteca and Union Island, situated 10 to 15 miles from the entrances of the three canyons.

On Foothills of the Salinas Valley—In the Salinas Valley, the favorable weeds in which the females deposit their eggs are not abundant, and the multiplication of the beet leafhopper occurs chiefly on beets; hence, higher percentages of infective overwintering leafhoppers should occur on the foothills of the Salinas Valley than on those of the San Joaquin Valley. Migrations of the spring-generation adults occur from the San Joaquin Valley to the foothills and cultivated areas of the Salinas Valley, as reported in a previous paper (*16*). The percentage of infectivity of the nymphs is more reliable than that of the adults during the spring in the Salinas Valley.

During 1925, 2,603 acres of beets had been grown near King City and 15,689 acres in the Salinas Valley, and a serious outbreak of sugar-beet curly top occurred that year. Sugar beets were grown near the base of the foothills east of San Ardo and on the Spreckels ranches near King City. The leafhoppers were commonly taken on *Erodium cicutarium* growing on the foothills west of San Ardo and near King City. A high percentage of infective overwintering adults and nymphs and adults of the spring generation occurred on the foothills, as shown in table 2.

During 1931, 278 acres of beets had been grown in the King City district and 1,304 acres in the Salinas Valley. As indicated in table 2, lower percentages of infective adults of the overwintering and spring generations occurred in 1932 in Pine Valley, situated between San Lucas and San Ardo.

During 1932, 1,050 acres of beets had been grown in the vicinity of King City and 8,508 acres in the Salinas Valley. The percentages of infective spring-brood adults collected in Hog Canyon near King City and on the foothills near Metz are given in table 2.

In Cultivated Areas.—After the 1925 outbreak of the beet leafhopper (*20*), overwintering adults were abundant in the beet fields of the Santa Clara and Salinas valleys. Planting of sugar beets was temporarily abandoned in these valleys during the season of 1925–26, owing to the fact that during the 1919 outbreak of the pest over one-half of the early

planted beets in the San Joaquin and Salinas valleys were infected with curly top by the overwintering females which remained in the cultivated areas (*10*).

Leafhoppers which remained in the cultivated areas during the winter were collected on February 23, 1926, in the beet fields from King City to Gonzales in the Salinas Valley, and 42 per cent were found to be infective. The insects probably invaded the beet fields from weeds growing in the cultivated areas: a large number of weeds (*17*) have been demonstrated to be naturally infected with curly top. A large flight of the adults of the spring generation occurred on April 19, and the next day 100 adults were collected on weeds growing in the cultivated areas near King City. Table 2 shows that 36 per cent of the leafhoppers were infective.

During 1932 the percentages of infective adults of the overwintering generation were higher than those of the spring generation in the Salinas Valley, as shown in table 2.

On Pasture Vegetation Germinating Early and Late.—The percentage of infectivity of the beet leafhopper may be associated with the time of germination of the seeds of the pasture vegetation by autumn or early-winter rainfall. When early-autumn rains germinate the seeds of host plants susceptible to curly top on the uncultivated plains and foothills at the time the flights occur from the cultivated areas, the opportunity for the spread of the disease is greater than during dry autumns or dry early winters, when the overwintering adults are forced to feed on perennial food plants, most of which are nonsusceptible to curly top. As more host plants become infected on the uncultivated plains and foothills, noninfective leafhoppers may pick up the virus and infective insects may become reinfected and thus their infective power may increase. The longer the insects are forced to feed on perennial noninfected food plants, the shorter the period of infectivity of the overwintering adults. It appears, then, if the infective power of the overwintering adults is reduced by feeding on perennial food plants, the spread of the disease to late-germinating pasture vegetation is limited, and hence a low percentage of infectivity of the adults of the spring generation should occur under natural conditions.

Many of the overwintering adults reared on curly-top beets in the northern San Joaquin Valley probably enter the northern canyons; and hence the percentages of infective beet leafhoppers in Little Panoche Pass, situated about 80 miles from the nearest beet fields, will be considered in relation to early and late germination of the seeds of the pasture vegetation. Most of the overwintering adults which fly into little

Panoche Pass breed on favorable weeds, such as Russian thistle (*Salsola kali* var. *tenuifolia*), bractscale (*Atriplex bracteosa*), and fogweed, or silverscale (*Atriplex argentea* subsp. *expansa*). As reported in a pre-

TABLE 3

Precipitation for the Autumn and Early Winter, Firebaugh and Mercy Hot Springs

(In inches)

Day	Firebaugh								Mercy Hot Springs			
	1923			1925			1928,* Nov.	1929, Dec.	1930–31†		1931, Nov.	1932, Nov.
	Sep.	Oct.	Nov.	Oct.	Nov.	Dec.			Nov.	Jan.		
1												
2						0.36						
3					0.02		0.05					
4					0.08							
5				0.03						0.60		
6				0.03						0.04		
7		0.15								0.58		
8												
9			0.06								0.18	
10			0.02	0.03				0.04				
11					0.02			0.07				
12				0.04	0.07			0.03				
13							1.30					
14							0.65				0.84	
15							0.15		0.02		0.07	
16								0.12	0.08			
17									0.02			
18						0.59						
19						0.18		0.02				
20								0.03				
21												
22	0.36									0.38		
23												
24												
25												
26	0.06								0.11		0.50	
27									0.02			
28												
29												
30			0.33									0.42
31												
Total	0.42	0.15	0.41	0.13	0.19	1.13	2.15	0.31	0.25	1.60	1.59	0.42

* Rainfall for October, trace.

† Rainfall for December, 0.00.

vious paper (*16*), migrations occur from the San Joaquin Valley to the foothills of the Salinas Valley, and hence the percentages of infectivity of the spring-generation adults in relation to early and late germination of the seeds of the pasture vegetation will not be discussed for the Salinas Valley.

The time of germination of pasture vegetation in Little Panoche Pass is of course related to the occurrence of the first rains. Table 3 shows the daily precipitation for the autumn and, in some years, for the early winter. No rainfall records are available during the years 1923, 1925, 1928, and 1929 at Mercy Hot Springs, situated in Little Panoche Pass, and hence the rainfall records at Firebaugh, situated in the valley floor about 25 miles from Mercy Hot Springs, are given.

During the first half of the autumn, about ½ inch of rainfall is sufficient to germinate the seeds of the pasture vegetation; during the last half of the autumn and early winter, when temperatures are lower and fogs are frequent, ¼ inch of rainfall is considered enough to germinate the seeds of the pasture vegetation. An examination of the daily precipitation (table 3) shows that for the years 1923, 1925, 1928, 1931, and 1932 there was sufficient rainfall for the seeds of the pasture vegetation to germinate during the autumn; and in Little Panoche Pass a high percentage of infective adults of the spring generation occurred (table 2). The rainfall records during 1929 and 1930–31 in table 3 show that there was not sufficient rainfall for the seeds of the pasture vegetation to germinate until winter; and in Little Panoche Pass the lowest percentages of infective adults of the spring generation occurred in these years (table 2).

There is one factor which may prevent the rapid autumnal spread of curly top to annual plants on the uncultivated plains and foothills. Young plants succumb much more rapidly to the disease than do older, deep-rooted plants. If the annual was young and infected with curly top by the overwintering female at the time of oviposition, the infected plant might die before the egg hatched, especially during the winter in the northern part of the San Joaquin Valley, when the egg period may require from 51 to 55 days (*15*). This of course diminishes the number of infective leafhoppers of the spring generation.

PERIOD OF INFECTIVITY DURING ADULT LIFE

Freitag (*4*) determined the period of infectivity during the adult life of the beet leafhopper, and the results are summarized in table 4. The outstanding result obtained with previously noninfective leafhoppers that fed for short periods on curly-top beets and were then transferred daily to healthy beets is the low percentages of infections obtained, as compared with those obtained from leafhoppers that fed for longer periods on diseased beets. Many of the infective leafhoppers apparently lost the capacity to produce infections during late adult life; others retained their infectivity but infected beets only at great intervals. The insect could be reinfected with the virus during late adult life; and it then

transmitted the virus as readily as recently molted adults. The evidence indicates that the curly-top virus does not multiply in the beet leafhopper and that the insects are merely internal mechanical carriers of the virus.

On Plants Immune to Curly Top.—Experiments were conducted to determine the ability of infective beet leafhoppers to transmit the curly-top virus after they had been transferred from diseased beets to immune

TABLE 4

SUMMARY OF RESULTS ON TRANSMISSION OF CURLY-TOP VIRUS BY SINGLE BEET LEAFHOPPERS FED FOR VARYING PERIODS ON A DISEASED BEET AND TRANSFERRED DAILY TO A HEALTHY BEET DURING ADULT LIFE*

Period on curly-top beets	Average number of beets inoculated	Beets infected		Average per cent of beets infected during					
		Average number	Average per cent	1–30 days	31–60 days	61–90 days	91–120 days	121–150 days	151–180 days
10, 20, 40, 60, 120, and 180 minutes	99.2	3.4	3.4	7.2	2.2	1.3	1.6	1.8	1.0
¼, ½, 1, 3, 7, 14, 21, and 28 days	130.7	15.1	11.6	26.5	15.0	5.6	3.9	0.5	0.0
Nymphal stages	132.2	15.6	11.8	31.9	14.8	5.2	1.2	0.0	0.8
	138.6	16.6	12.0	28.3	12.9	11.4	1.2	4.1	4.1
	160.4	10.7	6.7	18.7	7.9	3.3	1.8	1.7	5.7

* Summarized from: Freitag J. H. Negative evidence on the multiplication of curly-top virus in beet leafhopper, *Eutettix tenellus*. Hilgardia 10(9): 303–42. 1936.

breeding plants. Twenty large *Atriplex semibaccata* transplanted from the field were repeatedly inoculated by large numbers of infective leafhoppers, but all proved to be immune to curly top. Mammoth or Alameda sweet corn (*Zea Mays*) was also demonstrated to be immune to curly top (*21*).

Recently molted males which had completed the nymphal stages on diseased beets were transferred in lots of 50 to each *Atriplex semibaccata* and to each sweet corn plant. After the leafhoppers were kept on plants immune to curly top for a period of 30 days, 2 lots of 10 males were transferred singly to healthy sugar-beet seedlings for 1 day. The same procedure was repeated at the end of 60 days; but after 90 days, the number of insects was so reduced that 2 lots of 10 males were not always available. The results are indicated in table 5.

According to table 5, the average percentages of beets infected during successive 30-day periods by leafhoppers kept singly on beets for 1 day were as follows: *Atriplex semibaccata* 34.7, 14.1, and 6.7; sweet corn 27.3, 11.3, and 2.4. Corresponding figures obtained by Freitag (*4*) for sweet corn are as follows: 24.5, 6.8, and 3.1. There was a decrease in the percentages of beets infected during successive 30-day periods when the

insects were transferred singly from immune host plants and kept on beets for 1 day, which indicates that many of the infective leafhoppers lost the capacity to produce infection.

A comparison of the average percentages of beets infected during suc-

TABLE 5

TRANSMISSION OF CURLY-TOP VIRUS BY SINGLE INFECTIVE MALE BEET LEAFHOPPERS KEPT ON IMMUNE HOST PLANTS FOR PERIODS OF 30, 60, AND 90 DAYS AND ON HEALTHY BEETS FOR 1 DAY

Immune host plant	Beets infected of 10 inoculated		After 90 days on immune plants	
	After 30 days on immune plant	After 60 days on immune plant	Beets inoculated	Beets infected
Australian saltbush (fleshscale) (*Atriplex semibaccata*)				
No. 1	7	1	4	0
No 2	4	0	4	0
No. 3	1	1	4	0
No. 4	3	2	2	0
No. 5	3	3	1	1
No. 6	4	1	0	
No. 7	4	1	0	
No. 8	6	2	0	
No. 9	5	0	0	
No 10	5	2	0	
No. 11	4	1	0	
No. 12	4	2	0	
No. 13	3	1	0	
No. 14	3	2	0	
No. 15	2	1	0	
No. 16	1	2	0	
No 17	0	2	0	
Total	59	24	15	1
Average per cent	*34 7*	*14 1*		*6 7*
Alameda or Mammoth sweet corn (*Zea Mays*)				
No. 1	4	2	8	0
No. 2	3	0	10	0
No. 3	3	0	9	0
No. 4	3	1	3	0
No. 5	3	0	3	0
No. 6	2	0	8	1
No. 7	5	2	0	
No. 8	5	1	0	..
No. 9	4	2	0	..
No. 10	2	0	0	
No. 11	2	1	0	
No. 12	2	2	0	
No. 13	1	3	0	
No. 14	1	2	0	
No. 15	1	1	0	
Total	41	17	41	1
Average per cent	*27.3*	*11.3*		*2 4*

cessive 30-day periods as given in table 5, with the average percentages of infections during 1–30, 31–60, and 61–90 days obtained with leafhoppers which completed the nymphal stages on diseased beets as given in table 4 indicates that the juices from *Atriplex semibaccata* and sweet corn did not affect the period of infectivity of the beet leafhopper and probably had no effect on the curly-top virus.

When leafhoppers which completed the nymphal stages on diseased beets were provided with a healthy beet daily during adult life, or when infective adults were kept on immune plants, the highest percentages of infections were obtained during the first 30 days. The longer the overwintering adults are forced to feed on perennials nonsusceptible to curly top during dry autumns and dry early winters, the shorter is the period remaining for infectivity, and hence the spread of the disease by the overwintering adults to susceptible plants on the uncultivated plains and foothills would be reduced.

NATURAL HOST RANGE OF CURLY TOP AND FAVORABLE VIRUS RESERVOIRS

Annuals.—The natural host range of curly top and favorable virus reservoirs of the beet leafhopper in the cultivated areas and on the uncultivated plains and foothills have appeared in previous reports. Additional host plants are reported in this paper. Seventy-five species of plants in 48 genera belonging to 18 families have been reported in previous papers (*12, 13, 14, 17, 19, 22*) to be naturally infected with curly top. The annuals demonstrated to be naturally infected with curly top include 11 species of weeds on the uncultivated plains and foothills and 20 species of weeds or other wild plants in the cultivated areas (*17*). Seventeen species of annual economic plants and 11 species of annual ornamental plants were proved to be naturally infected with curly top, a total of 59 annuals.

Lessingia glandulifera (plate 2, *A*) and *Hemizonia virgata,* annual composits which serve as food plants of the beet leafhopper on the uncultivated plains and foothills, have been repeatedly tested but have not been found to be naturally infected with curly top. *L. glandulifera* transplanted from the field was not experimentally infected with curly top.

Biennials or Perennials.—After the return flights of the overwintering beet leafhoppers to the uncultivated plains and foothills during dry autumns, large numbers of leafhoppers congregate on perennials, most of which are not susceptible to curly top. Three perennials growing on the plains and foothills have been found to be naturally infected with curly top, but these are of minor importance as food plants of the adults

of the overwintering generation. The curly-top virus was repeatedly recovered from a naturally infected perennial—ballscale (*Atriplex fruticulosa*) (Chenopodiaceae)—during a period of six months, when the tests were discontinued. No similar tests were made with the other two species of naturally infected perennials—*Modiola caroliniana* (Malvaceae) and *Phacelia ramosissima* (Hydrophyllaceae).

The weeds and wild shrubs proved to be naturally infected with curly top in cultivated areas include 3 species that may be either annuals or biennials and 4 species of perennials. The virus was repeatedly recovered from one perennial—Mexican tea (*Chenopodium ambrosioides*) (Chenopodiaceae)—during a period of one year, after which no further tests were made. The perennial nightshade (*Solanum Douglassii*) (Solanaceae) was found to be naturally infected with curly top whenever a severe epidemic of the disease occurred; the virus apparently becomes more virulent at such a time and highly resistant weeds seem to become more susceptible to infection. The virus was recovered from 4 of 17 seedlings which were repeatedly inoculated by lots of 5 to 50 infective males. The virus was recovered from naturally infected perennial water smartweed (*Polygonium amphibium* var. *Hartwrightii*) and swamp smartweed (*P. Muhlenbergii*) (Polygonaceae), but no other tests were made with these weeds.

Among economic plants, potato (*Solanum tuberosum*) (Solanaceae), a herbaceous plant; Single or Plain parsley (*Petroselinum hortense*) (Umbelliferae), a biennial or short-lived perennial; and Hairy Peruvian alfalfa (*Medicago sativa*) (Leguminosae) and horse-radish (*Armoracia rusticana*) (Cruciferae), two perennials, were demonstrated to be naturally infected with curly top. The virus was rarely recovered from naturally infected horse-radish during the summer and not at all during the autumn, nor from cuttings grown from naturally infected horse-radish roots. No experiments have been conducted up to the present time to prove potato-tuber transmission of the virus or whether the virus could be recovered during the autumn and winter from parsley and alfalfa.

The ornamental flowering plants found to be naturally infected with curly top include 1 annual or short-lived perennial and 2 perennials. During the spring, the virus was transferred by previously noninfective beet leafhoppers from 6 of 7 naturally infected fish geraniums (*Pelargonium hortorum*) (Geraniaceae) to sugar beets, but during the autumn the virus was recovered from only 2 of the same plants. The perennial grass pink (*Dianthus plumarius*) (Caryophyllaceae) was demonstrated to be naturally infected with curly top, but no other tests were made.

Perennials Experimentally Infected.—Quailbrush, or lenscale (*Atriplex lentiformis*) (Chenopodiaceae), frequently grows 10 feet high and occurs on alkali flats and river benches. Six of 20 seedlings transplanted from the San Joaquin Valley were experimentally infected with curly top. In a previous experiment, 8 seedlings were experimentally infected with curly top, but one year later the virus was not recovered from the same plants. The 8 plants were then reinfected, and the virus was again transmitted by previously noninfective beet leafhoppers from only 2 of these to sugar beets.

Australian saltbush, or fleshscale (*Atriplex semibaccata*) (Chenopodiaceae), grown from seeds, showed a high degree of resistance to curly top; in fact, most seedlings were immune to the disease. Thirty seedlings were repeatedly inoculated with different lots of infective beet leafhoppers, but 27 of them could not be infected. The virus was not recovered a second time from 1 of 3 infected plants 23 days after the first recovery of the virus.

Atriplex semibaccata has not been found to be naturally infected with curly top. Some adults collected on *A. semibaccata* under natural conditions transmitted the curly-top virus to healthy sugar beets. On June 9, 1933, each of 50 adults of the spring generation captured on *A. semibaccata* growing on the foothills near Metz in the Salinas Valley was provided with a healthy beet seedling. It was found that 8 of 50 beets, or 16 per cent, developed typical symptoms of curly top. Some of the adults undoubtedly obtained the virus from other host plants of curly top growing on the foothills and, after the pasture vegetation became dry on the foothills, flew to *A. semibaccata* and retained the infective power.

In the Imperial, Salinas, and San Joaquin valleys, different lots of nymphs collected on large *Atriplex semibaccata* isolated from other breeding plants failed to transmit the curly-top virus to healthy sugar beets. On July 7, 1933, each of 50 nymphs collected on *A. semibaccata* growing on the foothills near Metz was provided with a healthy beet, but not a single case of curly top developed.

Nymphs bred on *Atriplex semibaccata* failed to obtain the curly-top virus even though infective adults were feeding on the same plants. In this experiment, 9 lots of 10 infective females oviposited for a period of 4 to 7 days in 9 large Australian saltbushes transplanted from the field. The females were then removed and played no further part in the experiment. Nine lots of 50 infective males which had completed the nymphal stages on diseased beets were confined in cages enclosing the saltbushes. Two hundred nymphs which hatched from eggs deposited in

the saltbushes were transferred in lots of 10 nymphs to 20 healthy beets, but not a single case of curly top developed.

Soap plant (*Chenopodium californicum*) (Chenopodiaceae) occurs in stream beds and on moist slopes of the foothills. The plants experimentally infected with curly top were grown from roots transplanted from the foothills of the Salinas Valley.

Alkali blite (*Suaeda moquini*) (Chenopodiaceae) grows commonly on

Fig. 1.—Spray equipment used to control the beet leafhopper on cattle spinach, or allscale (*Atriplex polycarpa*) growing in Tunney Gulch, San Joaquin Valley. This shrubby perennial saltbush is one of the most favorable food plants of the overwintering generation of beet leafhopper. It is green during dry autumns. (Courtesy, E. A. Schwing, Spreckels Sugar Company.)

alkali soil. The virus was recovered from 9 of 30 plants obtained in the San Joaquin Valley.

Tree tobacco (*Nicotiana glauca*) (Solanaceae), which was introduced from South America and occurs on the plains, in canyons, and in mountain passes of the San Joaquin Valley, was experimentally infected with curly top but has not been found to be naturally infected with the disease. The virus was recovered by previously noninfective beet leafhoppers from 6 of 10 experimentally infected seedlings and transferred to sugar beets. The overwintering leafhopper was rarely taken on *N. glauca* during dry autumns.

Perennials Nonsusceptible.—Two perennial saltbushes belonging to the family Chenopodiaceae serve as important food plants of the beet leafhopper during dry autumns. Large numbers of beet leafhoppers sometimes congregate on cattle spinach, or allscale (*Atriplex polycarpa*), a large perennial saltbush growing on the plains (fig. 1) in

mountain passes, and in canyons in the middle and southern San Joaquin Valley; but this shrub was not experimentally infected with curly top. Fifty-three small plants transplanted from the San Joaquin Valley were repeatedly inoculated by different lots of infective leafhopper, but the virus was not recovered by noninfective insects. In addition, 8 plants grown from seeds could not be experimentally infected with the disease.

Spinescale (*Atriplex spinifera*) (plate 1, *D*), another large perennial saltbush widely distributed on the uncultivated plains in the middle and southern San Joaquin Valley, also serves as a food plant of the leafhoppers during dry autumns, but 3 small plants transplanted from the field and 8 plants grown from seeds could not be experimentally infected with the disease.

Lepidospartum squamatum (Compositae) (plate 2, *B, C*) forms dense stands in the dry sandy stream beds of the Salinas River and its tributaries and in some of the mountain passes and canyons in the San Joaquin Valley. Owing to its wide distribution, it is one of the most important food plants of the beet leafhopper during dry autumns. Small plants removed from the field were not susceptible to curly top. All attempts to germinate the seeds were failures, hence the seedlings were not tested for experimental infection.

Gutierrezia californica (Compositae) (plate 1, *A*), a shrub from ¾ to ½ feet high, occurs on the uncultivated plains and foothills of the San Joaquin Valley. Overwintering adults are commonly taken on this plant before the seeds of the pasture vegetation germinate. Nine plants removed from the field were not susceptible to curly top.

Overwintering adults were commonly taken on *Haplopappus venetus* subsp. *vernonioides* (Compositae) (plate 2, *E*), a shrub from 1 to 2 feet high growing on the plains and foothills in the San Joaquin Valley. Twenty-six plants removed from the field and 21 plants grown from seeds were not susceptible to the disease.

Creek senecio (*Senecio Douglasii*) (Compositae) (plate 1, *B*), owing to its common occurrence, is a more important food plant of the overwintering beet leafhoppers in the Salinas than in the San Joaquin Valley. It is abundant in the Salinas River and tributaries and also occurs in dry stream beds, in canyons, and on the sandy or gravelly plains of the San Joaquin Valley. Twelve small plants removed from the field and 12 plants grown from seeds were not susceptible to curly top. The leaves were curled, and dark brown or black droplets of liquid (plate 2, *D*) exuded from the stems and leaves, but noninfective leafhoppers failed to recover the virus from the inoculated plants. Nymphs which hatched

from eggs deposited in *Senecio Douglasii* completed their life cycle in the greenhouse.

Alkali heath (*Frankenia grandifolia*) (Frankeniaceae) is common on the alkali plains and in canyons and mountain passes of the San Joaquin Valley and serves as a food plant of the overwintering beet leafhoppers during dry autumns. After the pasture vegetation becomes dry in the spring, males of the spring generation which do not fly into the cultivated areas were commonly taken on this plant. *F. grandifolia* on which beet leafhoppers were captured was not found to be naturally infected with curly top. The virus was not recovered by noninfective leafhoppers from plants on which repeated lots of infective insects had fed.

The overwintering beet leafhoppers were also taken on other perennials growing on the uncultivated plains and foothills during dry autumns, such as burro fat (*Isomeris arborea*) (Capparidaceae), buckbrush or chaparral (*Ceanothus cuneatus*) (Rhamnaceae), alkali mallow (*Sida hederacea*) (Malvaceae), common horehound (*Marrubium vulgare*) (Labatae), *Grindelia camporum* (Compositae), *Chrysopsis villosa* (Compositae), arrowweed (*Pluchea sericea*) (Compositae), and California sagebrush (*Artemisia californica*) (Compositae), but plants transplanted from the San Joaquin Valley were not susceptible to the disease. The overwintering adults have been taken on many other perennial species during dry autumns, but these have not been tested for natural and experimental infection with curly top.

RECOVERY OF CURLY-TOP VIRUS FROM IMPORTANT BREEDING PLANTS OF BEET LEAFHOPPER

During the past five years, attempts have been made to control the beet leafhopper, after the return flights from the cultivated areas to the uncultivated plains and foothills have occurred, by spraying perennials (fig. 1) on which leafhoppers congregate during dry autumns. The insecticide used consists of a mixture of 39 gallons of Diesel oil and 1 pound of pyrethrum extract. A second method of control of the beet leafhopper is by the destruction of the summer breeding plants near the foothills before the adults of the overwintering generation acquire the winged stage. One of the most important breeding plants, Russian thistle (*Salsola kali* var. *tenuifolia*), is destroyed by summer hoeing, dragging, or disking. The third method consists of burning Russian thistle during the autumn before the plants are torn loose and start rolling; this prevents the spread and reduces the seed supply. A more detailed report on the methods of control of the beet leafhopper has been published in a previous paper (*9*).

Although in the control program it was known that certain weeds are important breeding plants and also high populations of beet leafhoppers congregate on these weeds during the autumn flights, no information was at hand on the recovery of the virus from these infected weeds by single insects. Three species of annual saltbushes and Russian thistle serve as important breeding plants of the beet leafhopper in the cultivated areas. Bractscale (*Atriplex bracteosa*) is one of the most important breeding plants of the beet leafhopper, and high populations of the leafhopper of the summer and overwintering generations develop on this weed. Although large numbers of leafhoppers of the spring generation sometimes congregate on fogweed, or silverscale (*Atriplex argentea* subsp. *expansa*), and high populations of the summer generation develop, nevertheless this saltbush in most seasons is not as favorable for the development of large populations of the overwintering generation as *Atriplex bracteosa* and Russian thistle. Red orache, or redscale (*Atriplex rosea*), is a favorable host plant for the development of the leafhoppers of the summer generation but becomes dry too early for the development of the insects of the overwintering generation. Russian thistle is the most important breeding plant of the leafhopper, and high populations of the insects of the summer and overwintering generations occur on this weed.

Tests were made on recovering the curly-top virus with single previously noninfective males which were kept on the 4 species of infected weeds for periods of 2, 4, and 8 days, and also with single adults which completed the nymphal stages, requiring from 26 to 36 days in the greenhouse (*15*), on some of the same weeds. The 4 species of weeds were inoculated with the curly-top virus by 10 infective males for a period of 3 weeks or longer, and then the insects were removed from the weeds. Noninfective males, after feeding on each infected weed for periods of 2, 4, and 8 days, were transferred singly to healthy beet seedlings (table 6). The method usually adopted to obtain adults reared during the nymphal stages on infected weeds was to allow 10 infective females to oviposit in each species of weed for a period of only 4 days, so as to avoid high populations of nymphs, which drain the plants. To insure infection, 10 infective males were fed on the same weeds for 3 weeks and then were removed from the cages. After the nymphs which hatched from eggs deposited in each infected weed acquired the winged stage, each of 10 males was provided with a healthy beet (table 7).

Table 6 shows that single previously noninfective males which were kept on the 4 species of weeds for periods of 8 days produced higher average percentages of infections than single adults which fed on the infected weeds for 2 days. A comparison of the results obtained in tables 6 and

TABLE 6

INOCULATIONS OF HEALTHY BEET SEEDLINGS WITH CURLY-TOP VIRUS BY MEANS OF SINGLE PREVIOUSLY NONINFECTIVE BEET LEAFHOPPERS FED ON INFECTED WEEDS FOR PERIODS OF 2, 4, AND 8 DAYS

Infected weed on which insects fed	Insects on infected weeds, 2 days		Insects on infected weeds, 4 days		Insects on infected weeds, 8 days	
	Dates fed	Beets infected of 10 inoculated	Dates fed	Beets infected of 10 inoculated	Dates fed	Beets infected of 10 inoculated
Bractscale (*Atriplex bracteosa*)						
No. 1	Apr. 19–21	8	Apr. 19–23	10	Apr. 19–27	9
No. 2	Apr. 19–21	7	Apr. 19–23	7	Apr. 19–27	7
No. 3	Apr. 19–21	6	Apr. 19–23	9	Apr. 19–27	10
No. 4	Apr. 19–21	5	Apr. 19–23	9	Apr. 19–27	7
No. 5	Apr. 19–21	4	Apr. 19–23	9	Apr. 19–27	7
No. 6	Apr. 19–21	3	Apr. 19–23	5	Apr. 19–27	8
No. 7	May 7–9	0	May 7–11	2	May 7–15	1
No. 8	June 5–7	9	June 5–9	8	June 5–13	9
No. 9	June 5–7	8	June 5–9	8	June 5–13	8
No. 10	June 5–7	7	June 5–9	7	June 5–13	9
No. 11	June 5–7	7	June 5–9	8	June 5–13	6
No. 12	June 5–7	6	June 5–9	7	June 5–13	10
Total		70		89		91
Average per cent		*58.3*		*74.2*		*75.8*
Fogweed (silverscale) (*Atriplex argentea* subsp. *expansa*)						
No. 1	Apr. 19–21	7	Apr. 19–23	10	Apr. 19–27	8
No. 2	Apr. 19–21	7	Apr. 19–23	10	Apr. 19–27	6
No. 3	Apr. 19–21	7	Apr. 19–23	8	Apr. 19–27	9
No. 4	Apr. 19–21	6	Apr. 19–23	8	Apr. 19–27	7
No. 5	Apr. 19–21	4	Apr. 19–23	9	Apr. 19–27	5
No. 6	Apr. 19–21	2	Apr. 19–23	7	Apr. 19–27	8
No. 7	May 7–9	8	May 7–11	6	May 7–15	8
No. 8	May 7–9	5	May 7–11	3	May 7–15	4
No. 9	May 7–9	3	May 7–11	2	May 7–15	6
No. 10	May 7–9	3	May 7–11	1	May 7–15	1
No. 11	May 7–9	2	May 7–11	7	May 7–15	6
No. 12	May 7–9	1	May 7–11	2	May 7–15	5
Total		55		73		73
Average per cent		*45.8*		*60.8*		*60.8*
Red orache (redscale) (*Atriplex rosea*)						
No. 1	Apr. 19–21	9	Apr. 19–21	8	Apr. 19–27	9
No. 2	Apr. 19–21	8	Apr. 19–21	9	Apr. 19–27	9
No. 3	Apr. 19–21	8	Apr. 19–21	8	Apr. 19–27	8
No. 4	Apr. 19–21	6	Apr. 19–21	8	Apr. 19–27	9
No. 5	Apr. 19–21	6	Apr. 19–21	8	Apr. 19–27	8
No. 6	May 7–9	7	May 7–11	7	May 7–15	7
No. 7	May 7–9	7	May 7–11	3	May 7–15	6
No. 8	May 7–9	6	May 7–11	1	May 7–15	3
No. 9	May 7–9	4	May 7–11	6	May 7–15	4
No. 10	May 7–9	4	May 7–11	4	May 7–15	4
No. 11	May 7–9	3	May 7–11	4	May 7–15	6
No. 12	June 5–7	3	June 5–9	4	June 5–13	3
Total		71		70		76
Average per cent		*59.2*		*58.3*		*63.8*

TABLE 6—(*Concluded*)

Infected weed on which insects fed	Insects on infected weeds, 2 days		Insects on infected weeds, 4 days		Insects on infected weeds, 8 days	
	Dates fed	Beets infected of 10 inoculated	Dates fed	Beets infected of 10 inoculated	Dates fed	Beets infected of 10 inoculated
Russian thistle (*Salsola kali* var. *tenuifolia*)						
No. 1	May 3–5	0	May 3–7	4	May 3–11	5
No. 2	May 5–7	2	May 5–9	1	May 5–13	8
No. 3	May 7–9	1	May 7–11	1	May 7–15	2
No. 4	June 5–7	7	June 5–9	5	June 5–13	8
No. 5	June 5–7	7	June 5–9	5	June 5–13	5
No. 6	June 5–7	6	June 5–9	5	June 5–13	5
No. 7	June 5–7	2	June 5–9	2	June 5–13	1
No. 8	June 5–7	1	June 5–9	2	June 5–13	9
No. 9	June 5–7	0	June 5–9	4	June 5–13	4
No. 10	June 28–30	0	June 28–July 2	1	June 28–July 6	1
No. 11	July 17–19	7	July 17–21	7	July 17–25	8
No. 12	July 17–19	5	July 17–21	2	July 17–25	3
No. 13	July 17–19	2	July 17–21	1	July 17–25	3
No. 14	July 17–19	1	July 17–21	2	July 17–25	1
No. 15	July 17–19	1	July 17–21	1	July 17–25	2
No. 16	July 17–19	1	July 71–21	0	July 17–25	5
No. 17	July 17–19	0	July 17–21	5	July 17–25	2
No. 18	July 17–19	0	July 17–21	3	July 17–25	3
Total		43		51		75
Average per cent		23.9		28.3		41.7

7 shows that single males which completed the nymphal stages on the 3 species of infected saltbushes produced the highest percentages of infections.

According to table 6, single previously noninfective males were not able to recover the virus often from some of the infected weeds, as illustrated by *Atriplex bracteosa* no. 7, *A. argentea* subsp. *expansa* no. 10, and many Russian thistles; also when the nymphal stages were completed on Russian thistle (table 7). It is evident that some Russian thistles are resistant to the curly-top virus, while from some plants the virus can be recovered very readily by single adults reared during the nymphal stages on them.

Previously noninfective males recovered the virus from the 3 species of saltbushes monthly during the season's duration of the weeds and transferred it to sugar beets. The insects recovered the virus after the stems became woody and yellow, with the older leaves dry but the younger ones still green.

Russian thistles grown from seeds have been experimentally infected with the curly-top virus, and the virus was also recovered from small

TABLE 7

Inoculations of Healthy Beet Seedlings with Curly-Top Virus by Means of Single Beet Leafhoppers which Completed the Nymphal Stages on Infected Weeds

Infected weed on which insects fed	Dates adults were transferred from infected weeds to beets	Beets infected of 10 inoculated
Bractscale (*Atriplex bracteosa*)		
No. 13	May 8	10
No. 14	May 8	8
No. 15	May 8	8
No. 16	May 8	6
No. 17	May 8	5
No. 18	May 8	5
No. 19	May 8	4
No. 1	June 1	7
No. 2	June 1	10
No. 3	June 1	9
No. 4	June 1	8
No. 5	June 1	10
No. 6	June 1	8
No. 20	July 11	10
No. 21	Sep. 29	8
No. 22	Oct. 1	10
No. 23	Oct. 4	8
No. 24	Oct. 10	9
Total		143
Average per cent		*79.4*
Fogweed (silverscale) (*Atriplex argentena* subsp *expansa*)		
No. 1	June 1	8
No. 2	June 1	10
No. 3	June 1	7
No. 4	June 1	8
No. 5	June 1	8
No. 6	June 1	6
No. 13	July 19	10
No. 14	Sep. 29	10
Total		67
Average per cent		*83.7*
Red orache (redscale) (*Atriplex rosea*)		
No. 13	May 8	7
No. 14	May 8	5
No. 15	May 8	4
No. 16	May 8	3
No. 17	May 8	2
No. 18	July 20	10
No. 19	July 21	9

Infected weed on which insects fed	Dates adults were transferred from infected weeds to beets	Beets infected of 10 inoculated
Red orache (redscale) (*Atriplex rosea*)—Continued		
No. 20	July 23	10
No. 21	July 23	10
No. 22	July 26	10
No. 23	Sep. 1	3
No. 24	Oct. 5	7
Total		80
Average per cent		*66.7*
Russian thistle (*Salsola kali* var. *tenuifolia*)		
No. 19	May 8	7
No. 20	May 8	5
No. 21	May 8	3
No. 22	May 17	10
No. 23	May 17	6
No. 24	May 17	4
No. 25	May 17	2
No. 26	May 17	2
No. 27	May 18	5
No. 28	May 22	5
No. 29	May 22	5
No. 30	May 22	4
No. 31	May 22	2
No. 32	May 22	2
No. 33	May 22	1
No. 34	May 31	10
No. 35	May 31	9
No. 36	May 31	7
No. 37	May 31	1
No. 38	May 31	1
No. 39	July 20	1
No. 40	July 27	2
No. 41	Aug. 7	3
No. 42	Aug. 7	3
No. 43	Aug. 7	2
No. 44	Aug. 7	1
No. 45	Aug. 8	5
No. 46	Aug. 8	1
No. 47	Sep. 23	1
No. 48	Oct. 5	6
Total		116
Average per cent		*38.7*

naturally infected plants during the 1919 outbreak of the beet leafhopper. During 1933, attempts were made to experimentally infect with the curly-top virus thrifty-growing Russian thistles (plate 3, *A*) with long internodes and linear leaves (plate 3, *B*) transplanted from sandy soil near Manteca on May 11, and stunted plants with short internodes, thick stems, and prickle-tipped leaves (plate 3, *C*) collected on the plains in the middle San Joaquin Valley. Thirteen plants were inoculated with the curly-top virus by lots of 10 to 20 infective leafhoppers for periods varying from 16 to 42 days. Repeated lots of 20 previously noninfective males were fed on the plants for 2 days and then each lot of insects was divided among 2 healthy beet seedlings. The virus was recovered from 5 of 8 thrifty-growing but not from 5 stunted plants.

The fact that Russian thistles with long internodes are more susceptible to curly top than stunted plants with short internodes is important because higher populations of beet leafhoppers usually occur on the former. In another experiment, a total of 69 Russian thistles were inoculated with the curly-top virus by infective leafhoppers during different seasons of the year; the virus was recovered by previously noninfective adults from 56 plants, while 13 plants, or 18.8 per cent, were resistant or immune.

Tests were made to determine the percentage of infective nymphs on Russian thistles under natural conditions. One hundred nymphs were collected on September 7, 1933 on large Russian thistles about 10 miles southwest of Modesto in the San Joaquin Valley, and 9 per cent transmitted the curly-top virus to sugar beets. Another test was made with 50 nymphs collected on the same date on Russian thistles isolated from other breeding plants near Nile Garden in the San Joaquin Valley, but not a single nymph transmitted the virus to sugar beets.

Bassia hyssopifolia is a favorable breeding plant of the summer generation of beet leafhopper. Fifty nymphs were collected on September 6, 1933, on tall plants near Gustine in the San Joaquin Valley, and each was provided with a healthy beet; but not a single case of curly top developed. Small plants removed from the field were found to be immune to curly top: 7 plants were each inoculated by lots of 10 infective males for periods varying from 18 to 28 days, but 7 lots of 20 noninfective males failed to recover and transfer the virus to 14 sugar beets. Fifty males and 80 nymphs bred on 2 plants inoculated by 10 infective females for periods varying from 38 to 54 days failed to recover the virus. It is evident that leafhoppers bred on plants immune to curly top are noninfective even though infective females were feeding on the plants during the egg and nymphal stages.

WEEDS WHICH ATTENUATE THE CURLY-TOP VIRUS

Carsner and Stahl (*3*) reported that by passage of the curly-top virus through sowbane, or nettleleaf goosefoot (*Chenopodium murale*) (plate 4, *A, B, C*) the virus was so attenuated that when transmitted to healthy

TABLE 8

INNOCULATIONS OF HEALTHY BEET SEEDLINGS WITH CURLY-TOP VIRUS BY MEANS OF SINGLE AND LOTS OF 10 BEET LEAFHOPPERS REARED ON WEEDS WHICH ATTENUATE THE VIRUS

Infected weeds on which insects fed	Dates adults were transferred from infected weeds to beets	Single-insect inoculations			Inoculation by lots of 10 insects		
		Beets inoculated	Beets infected		Beets inoculated	Beets infected	
			Number	Per cent		Number	Per cent
Sowbane (nettleleaf goosefoot) (*Chenopodium murale*)							
No. 1	June 4	30	1	3.3	0		.
No. 2	June 30	0			2	0	0.0
	Sep. 25*	10	0	0.0	10	1	10.0
No 3.	Aug. 2–3	10	3	30.0	8	5	62.5
No. 4	Aug. 29–Sep. 1	10	0	0.0	1	1	100.0
	Nov. 9–10*	10	3	30.0	2	0	0.0
No. 5	Aug. 29–Sep. 1	10	1	10.0	1	1	100.0
No. 6	Sep. 1–5	10	1	10.0	1	1	100.0
	Nov. 9–24*	10	2	20.0	4	4	100.0
No. 7	Sep 25	10	1	10.0	10	0	0.0
No. 8	Sep. 25–Oct. 2	10	1	10.0	10	0	0.0
No. 9	Oct. 2	10	0	0.0	10	1	10.0
No. 10	Oct. 31–Nov. 13	10	1	10.0	6	3	50.0
No. 11	Nov. 1–15	10	0	0.0	10	4	40.0
No. 12	Nov. 6–Dec. 3	10	0	0.0	6	1	16.7
No. 13	Nov. 17–24	10	0	0.0	1	1	100.0
No. 14	Nov. 17–24	10	2	20.0	1	0	0.0
Total or *Average*		180	16	8.9	83	23	27.7
Lamb's-quarters (white pigweed) (*Chenopodium album*)							
No. 1	June 4–11	10	2	20.0	1	1	100.0
No. 2	June 4–11	10	1	10.0	3	3	100.0
No. 3	June 4–11	10	1	10.0	2	0	0.0
No. 4	June 4–21	10	1	10.0	1	1	100.0
No. 5	July 17–24	10	4	40.0	10	7	70.0
No. 6	Aug. 3	10	1	10.0	5	0	0.0
No. 7	Sep 5	10	4	40.0	1	1	100.0
No. 8	Sep 5–20	10	9	90.0	1	1	100.0
No. 9	Sep. 24	10	1	10.0	10	0	0.0
No. 10	Sep. 28	10	1	10.0	10	1	10.0
No. 11	Oct. 31	10	4	40.0	0		.
Total or *Average*		110	29	26.4	44	15	34.1

TABLE 8—(*Concluded*)

Infected weeds on which insects fed	Dates adults were transferred from infected weeds to beets	Single-insect inoculations			Inoculation by lots of 10 insects		
		Beets inoculated	Beets infected		Beets inoculated	Beets infected	
			Number	Per cent		Number	Per cent
Alkali blite (*Suaeda moquini*)							
No. 1	July 9	0			4	1	25.0
	Sep. 4–Oct. 11*	10	0	0.0	0		
No. 2	July 9	0			1	1	100.0
No. 3	July 17–23	10	2	20.0	4	0	0.0
	Oct. 31*	5	0	0.0	0		
No. 4	Aug. 4	10	2	20.0	2	0	0.0
	Aug. 9–Sep. 4	10	0	0.0	2	0	0.0
No. 5	Oct. 23	10	0	0.0	2	1	50.0
No. 6	Oct. 23	10	3	30.0	0		
No. 7	Oct. 31	10	2	20.0	0		
No. 8	Nov. 2	10	1	10.0	0		
No. 9	Nov. 5	10	1	10.0	0		
Total or *Average*		95	11	11.6	15	3	20.0
Curly dock (*Rumex crispus*)							
No. 1	Aug. 15	10	0	0.0	5	1	20.0
No. 2	Aug. 29–Sep. 5	10	0	0.0	1	1	100.0
No. 3	Aug. 29–Sep. 5	10	2	20.0	1	0	0.0
	Oct. 1*	0			5	4	80.0
No. 4	Aug. 29–Sep. 20	20	1	5.0	1	1	100.0
	Nov. 14–22*	10	0	0.0	4	2	50.0
No. 5	Sep. 5–29	10	0	0.0	1	0	0.0
	Oct. 31–Nov. 28	10	0	0.0	3	2	66.7
No. 6	Sep. 5	10	0	0.0	1	1	100.0
	Oct. 31–Nov. 3*	10	0	0.0	1	0	0.0
	Nov. 9–24*	0			8	1	12.5
No. 7	Oct. 2–5	10	3	30.0	9	1	11.1
No. 8	Oct. 2–5	10	2	20.0	4	4	100.0
No. 9	Oct. 5	10	1	10.0	10	1	10.0
No. 10	Oct. 5	10	1	10.0	0		
No. 11	Oct. 5	10	0	0.0	1	1	100.0
No. 12	Oct. 5–10	10	2	20.0	3	3	100.0
No. 13	Oct. 6	10	5	50.0	3	2	66.7
No. 14	Oct. 9	10	0	0.0	5	4	80.0
No. 15	Oct. 9	10	2	20.0	7	7	100.0
No. 16	Oct. 9	10	4	40.0	2	2	100.0
No. 17	Oct. 9	10	3	30.0	5	4	80.0
No. 18	Oct. 10	10	1	10.0	2	0	0.0
Total or *Average*		220	27	12.3	82	42	51.2

* Second-brood adults.

young sugar beets it either failed to cause the disease or usually produced only mild cases.

Lackey (*7*) confirmed the previous work with *Chenopodium murale.* He reported that 2 leafhoppers with attenuated virus infected 8 of 22

beets inoculated, or 36 per cent; and 10 leafhoppers with attenuated virus infected 21 of 38 beets, or 55 per cent. Lackey (*8*) also found that lamb's-quarters, or white pigweed (*Chenopodium album*), attenuates the curly-top virus.

Carsner (*2*) found two perennials—alkali blite (*Suaeda moquini*) (plate 1, *C*) and curly dock (*Rumex crispus*)—that are resistant to the action of the curly-top virus and attenuate the virus when it is passed through them.

An experiment somewhat similar to the one performed by Lackey was conducted to determine the percentage of single adults and lots of 10 adults that could recover and transfer the virus to beets from the 4 species of experimentally infected weeds which attenuate the virus. Twenty-five infective female leafhoppers inoculated each weed with the curly-top virus and deposited eggs in each plant during a period of 10 days, and then the females were removed. Sometimes two broods of adults were reared on the same weed, the plant again being exposed to 25 infective females. The adults of each brood were transferred singly to 10 beets or in lots of 10 adults to 1 beet. The results obtained with weeds which attenuate the virus are indicated in table 8, but weeds from which the virus was not recovered are omitted.

A detailed comparison of the average percentages of beets infected cannot be made in table 8, since a larger number of beets were used with single insects than with lots of 10 insects. A compilation of the data shows that single insects tested with 10 beets did not transfer the virus so often as 10 adults on 1 beet.

A few points of interest may be summarized from table 8 as follows:

Chenopodium murale no. 2: Adults of the first brood produced no infections, while 10 lots of 10 insects of the second brood infected 1 of 10 beets.

Chenopodium murale no. 3: Single adults infected 3 of 10 beets and 5 lots of 10 insects infected 5 beets on August 2, but on August 3, 3 lots of 10 insects failed to produce infections.

Chenopodium murale nos. 7 and 8: Single adults infected 1 of 10 beets, but 10 lots of 10 leafhoppers failed to produce infections.

Chenopodium murale no. 9: Single adults failed to infect 10 beets while 10 lots of 10 insects infected 1 of 10 beets.

Adults reared on many of the weeds inoculated with the curly-top virus failed to recover the virus, which indicates that such weeds were highly resistant or immune. The virus was recovered from 14 of 34 *Chenopodium murale,* 11 of 15 *C. album,* 9 of 30 *Suaeda moquini,* and 19 of 37 *Rumex crispus* plants.

Chenopodium album was not a favorable breeding plant, and usually low populations of adults were reared, but on an occasional plant large numbers of adults were obtained. On 5 *C. album* plants, the nymphs failed to acquire the winged stage. *Suaeda moquini* was also an unfavorable weed to rear nymphs; the nymphal stages were prolonged and usually low populations of adults were obtained.

SUMMARY

Higher percentages of infective adults of the spring generation usually occurred in the northern canyons of the San Joaquin Valley than in Little Panoche Pass, situated in the middle San Joaquin Valley about 80 miles away from the nearest beet fields. In all probability many overwintering adults reared on curly-top beets flew into the northern canyons and spread the virus to susceptible plants, and hence higher percentages of infective adults of the spring generation resulted.

There appears to be a correlation between the percentages of infective beet leafhoppers of the spring generation and early or late germination of the seeds of the pasture vegetation by autumn or early-winter rainfall in Little Panoche Pass: for example, during five years when there was rainfall in November, the infectivity varied from 16 to 42 per cent; during two years with December or January rainfall, the infectivity varied from 2 to 6 per cent.

There was a decrease in the percentage of beets infected during successive 30-day periods by adults kept on plants immune to curly top and transferred singly to beets for 1 day. Many of the infective beet leafhoppers apparently lost the capacity to produce infection. The evidence indicates that the juices from the immune host plants did not affect the period of infectivity of the leafhoppers and probably had no effect on the curly-top virus.

With the additions reported in this paper, seventy-five species of plants in 48 genera belonging to 18 families, including 59 species of annuals, 1 annual or short-lived perennial, 3 biennials, 1 biennial or short-lived perennial, and 11 perennials, have been demonstrated to be naturally infected with curly top.

Three species of perennials growing on the uncultivated plains and foothills were found to be naturally infected with curly top, but 16 species of perennials which serve as food plants of the beet leafhopper during dry autumns and early winters were not susceptible to the disease. The longer the overwintering adults are forced to feed on perennials nonsusceptible to curly top during dry autumns and early winters on the uncultivated plains and foothills, the shorter is the period remain-

ing for infectivity; and hence the less the spread of the disease to susceptible annuals after their seeds germinate.

The virus was repeatedly recovered from ballscale (*Atriplex fruticulosa*) during a period of six months and from Mexican tea (*Chenopodium ambrosioides*) during a period of one year, after which no further tests were made.

Natural infection of the perennial nightshade (*Solanum Douglassii*) was found during severe epidemics of the disease and may be associated with increased virulence of the virus.

The curly-top virus was recovered from naturally infected fish geranium (*Pelargonium hortorum*) more often during the spring than during the autumn. This may be associated with a higher concentration of the virus during the spring.

Perennial seedlings, such as quailbrush, or lenscale (*Atriplex lentiformis*), and Australian saltbush, or fleshscale (*A. semibaccata*), showed a high degree of resistance to the disease. The virus was recovered from infected seedlings of *A. lentiformis*, but one year later the virus was not recovered from these same plants. When these plants were reinfected, the virus was recovered from 2 of 8 plants. Seedlings of *A. semibaccata* were rarely infected with curly top, and large old plants were immune. The virus was not recovered from 1 of 3 infected seedlings 23 days after the first recovery of the virus.

The recovery of the virus from weeds which serve as the most important breeding plants of the beet leafhopper obtained with single noninfective leafhoppers which fed on the infected weeds for periods of 2, 4, and 8 days, and with single adults which completed the nymphal stages on the infected weeds is given in tables 6 and 7. The most favorable virus reservoir was *Atriplex bracteosa,* followed by *A. argentea* subsp. *expansa*, then *A. rosea* and lastly Russian thistle. The virus was recovered from all infected plants of the 3 species of saltbushes, but 15 of 69, or 18.8 per cent, of the Russian thistles were immune.

The virus was recovered monthly by previously noninfective beet leafhoppers from the 3 species of infected saltbushes during the season's duration of the plants and transferred to beets.

The transmission of the curly-top virus by single and by lots of 10 adults reared on 4 species of infected weeds which attenuate the virus is shown in table 8. Adults reared on the 4 species of weeds inoculated with the curly-top virus frequently failed to recover the virus.

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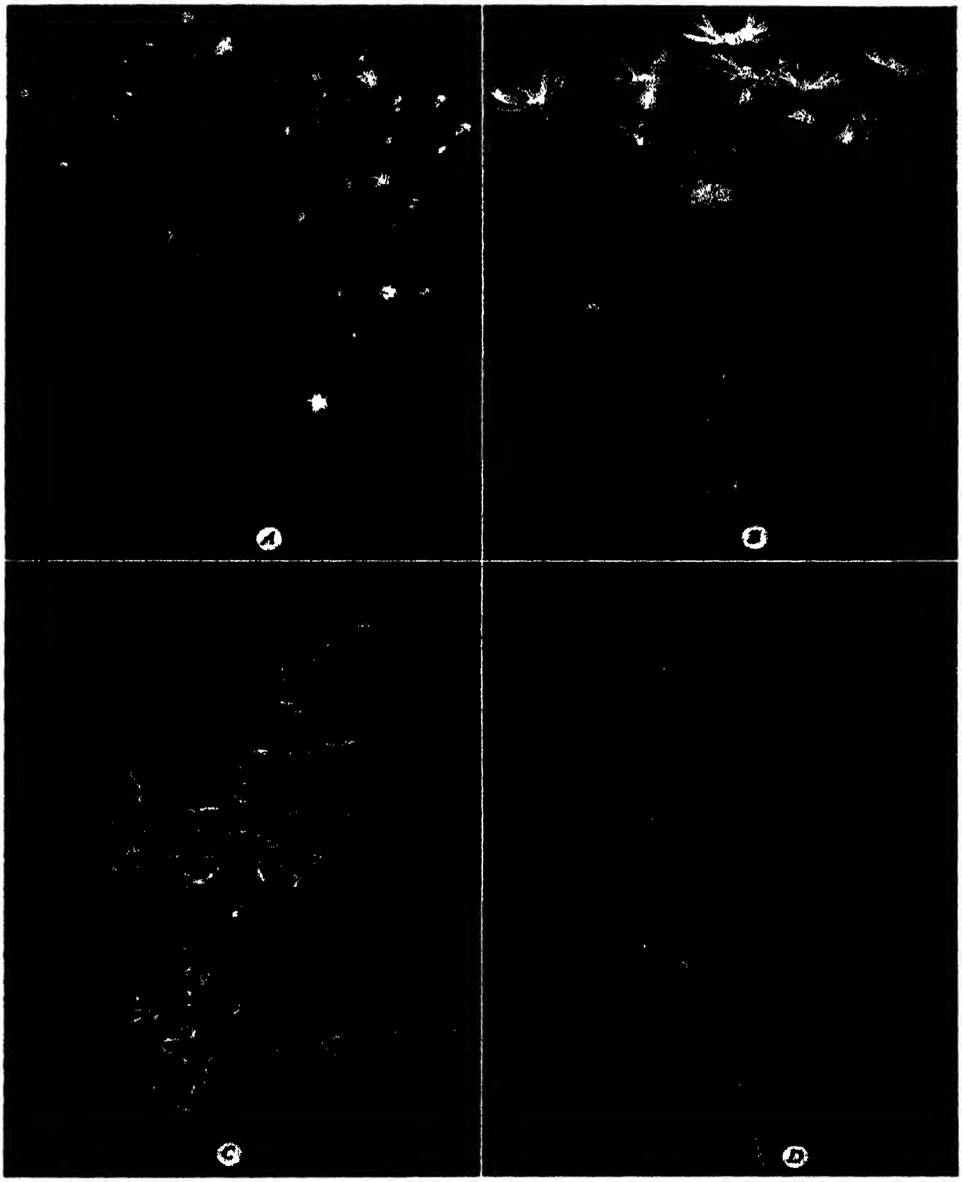

Plate 1.—*A*, Branch of *Gutierrezia californica* showing linear leaves and disk flowers. This perennial shrub occurs on the uncultivated plains and foothills of the San Joaquin Valley, and overwintering adults are commonly taken on this plant during dry autumns. *B*, Branch of creek senecio (*Senecio Douglasii*) showing narrow lobed leaves and clusters of flowers. This perennial, owing to its common occurrence, is a more important food plant of the overwintering adults during dry autumns in the Salinas than in the San Joaquin Valley. (Compare with plate 2, *D*.) *C*, Branch of alkali blite (*Suaeda moquini*) showing cylindrical leaves. The curly-top virus is attenuated by passage through this plant. *D*, Branch of spinescale (*Atriplex spinifera*) showing spines bearing sessile leaves. This large perennial saltbush is widely distributed on the uncultivated plains and foothills in the middle and southern San Joaquin Valley and serves as a food plant of the adults of the overwintering generation during dry autumns.

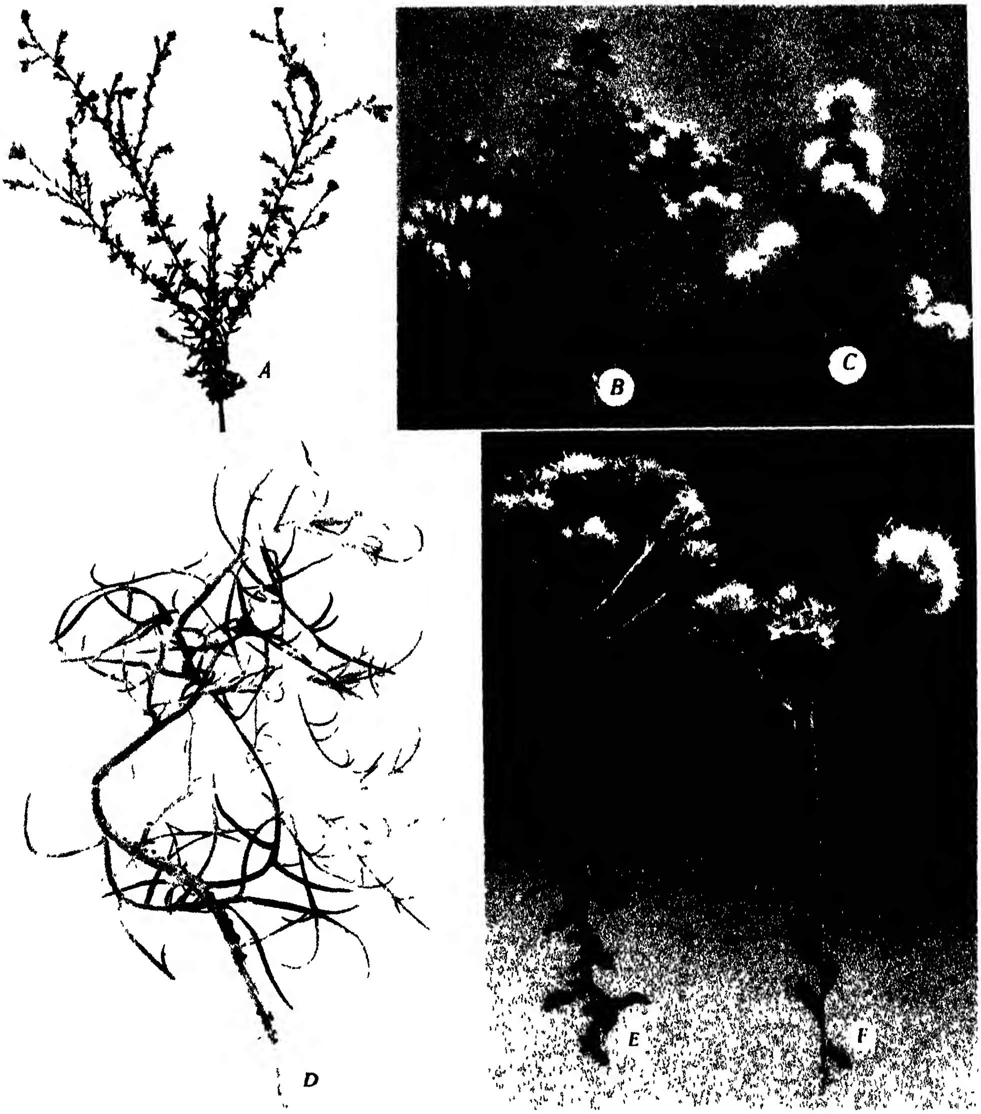

Plate 2 —*A*, *Lessingia glandulifera* showing erect branched stems bearing ovate or linear leaves and flowers This annual serves as a food plant of the overwintering adults on the uncultivated plains and foothills during dry autumns It has not been found to be naturally infected and was not experimentally infected with curly top. *B* and *C*, *Lepidospartum squamatum* *B*, branch showing flowers and scalelike leaves, *C*, branch showing bristles arising from seeds. *D*, Branch of creek senecio (*Senecio Douglasii*) showing curled leaves and black droplets of liquid exuding from the stem; but noninfective leafhoppers failed to recover the virus from the inoculated plants. (Compare with plate 1, *B*.) *E* and *F*. *Haplopappus venetus* subsp. *vernonioides* *E*, branch showing serrated, sessile leaves and clusters of flowers, *F*, branch showing silky pubescent arising from seeds. The overwintering adults were commonly taken on this perennial shrub growing on the uncultivated plains and foothills of the San Joaquin Valley during dry autumns.

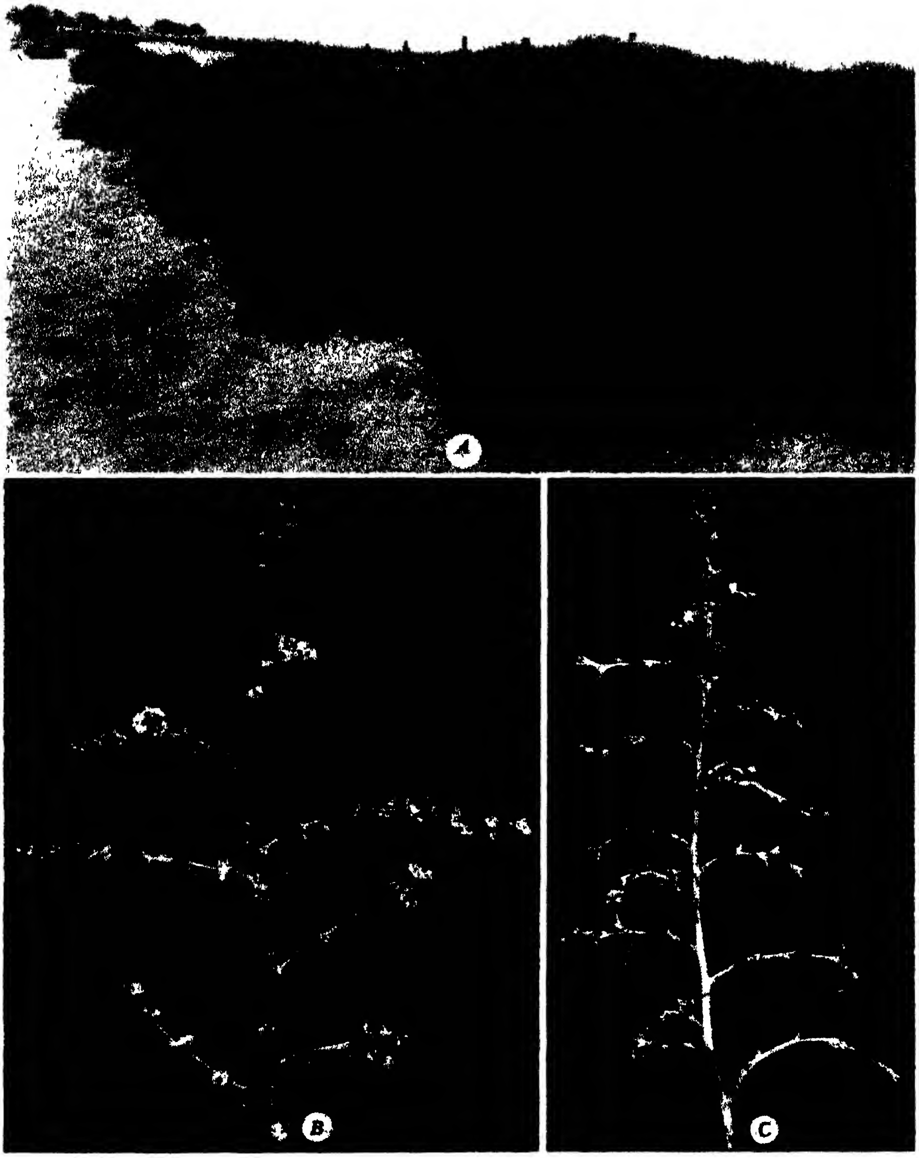

Plate 3.—Russian thistle (*Salsola kali* var. *tenuifolia*): *A*, plants growing in sandy soil along the roadside; *B*, branch from a large plant showing long internodes and linear leaves; *C*, branch from a stunted plant showing short internodes, thick stems, and prickle-tipped leaves. The leafhoppers multiply in enormous numbers on this plant in the interior regions of the state since it remains succulent from spring to autumn.

Plate 4.—Sowbane, or nettleleaf goosefoot (*Chenopodium murale*): *A*, secondary shoot from a plant experimentally infected with curly top showing curled apical leaves; *B*, branch and leaf from a healthy plant; *C*, plant experimentally infected with curly top showing secondary shoots with dwarfed leaves. The older leaves were removed from the middle portion of the plant The virus is attenuated by passage through this weed and back to sugar beets

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BREEDING FOR RESISTANCE TO ONION DOWNY MILDEW CAUSED BY PERONOSPORA DESTRUCTOR[1]

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(Results of a coöperative investigation conducted by the United States Department of Agriculture Bureau of Plant Industry and the California Agricultural Experiment Station.)

INTRODUCTION

THE ONION DOWNY-MILDEW FUNGUS, *Peronospora destructor* (Berk.) Casp., is practically world-wide in distribution (*2, 5*).[5] It was first described in 1841 by Berkeley (*1*) in England, and reported by Trelease (*12*) from Wisconsin in 1884. Subsequently it has been found in a number of other states and has frequently assumed epidemic proportions.

Because adequate control measures have not been developed, the disease continues to cause losses of varying magnitude. Such losses are usually most severe under conditions of moderate temperature and high humidity.

Even though recent work by Yarwood (*14, 15*) and by McWhorter and Pryor (*7*) indicates the fungicidal efficacy of certain chemical mixtures, the more satisfactory means of prevention involves the development of disease-resistant varieties. In this paper are presented data relative to varietal reaction to mildew, discussion of resistant types that have been found, and the present status of the breeding work designed to transmit resistance to varieties of commercial importance. The studies were made at Davis, California, and at other experimental tracts, as mentioned in the text.

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[5] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

PREVALENCE AND DESTRUCTIVENESS IN THE UNITED STATES

Downy mildew, though sporadic in occurrence, is probably the most destructive disease of onions in the United States. It has been abundant at times in each of the principal bulb-producing states, with the exception of Texas, and in each of the seed-producing states with the exception of Idaho. The disease demanded attention in Massachusetts, New York, Michigan, Oregon, and California during the eleven years from 1928 to 1938. The work in the states has been generally focused on host-parasite relations, on factors contributing to or aiding infection, and on fungicidal control. The disease frequently spreads rapidly and ruins large acreages, becoming most prevalent under conditions of high humidity.

On the crops grown for green bunching or for bulbs, downy-mildew infection may significantly reduce the quality and yield of the crop but rarely, if ever, causes complete loss. In New York, Cook (*2*) reported that bulb enlargement is significantly reduced if the plants become infected when small. In early 1938, at Davis, Sacramento, and Milpitas, in California, many plants of the extremely susceptible varieties Yellow Bermuda and Crystal White Wax were killed by mildew before the bulbs were one-fourth grown. Obviously the injury to the bulb crop is due primarily to killing of the foliage and consequent reduction in the size of the mature bulbs. Although bulbs are occasionally invaded by the mycelium, direct injury from bulb infection does not appear to be serious in America. In crops grown for bulbs or bunching, infected plants may survive and produce a fair crop even after a severe attack on the foliage.

In California the disease is particularly serious in the crop grown for seed. Total loss of seed fields has been observed. Frequently, satisfactory yields are obtained even though the leaves are killed by mildew, but if the seedstalks are severely infected the seed yield is reduced. Mother bulbs are planted from September to January, according to the variety; and, since the seed does not mature until July or August, the foliage is frequently exposed to infection for a period of six months. Normally considerable rainfall, fog, and dew occur from December to April; these conditions combined with favorable temperatures provide ideal conditions for infection, sporulation, and spread of the fungus. As a rule, in the interior valleys conditions become unfavorable for mildew after this time because the humidity decreases and the temperature increases. Along the coast, however, conditions may remain favorable for spread

of mildew considerably longer. Seedstalks usually appear after February 15, the time varying with the variety and date of planting. Obviously, decreases in seed yield are determined by the time and resulting severity of infection. The disease in California, during the last nineteen years, has caused losses as high as 60 to 80 per cent (table 1). In some

TABLE 1

ESTIMATED LOSSES DUE TO DOWNY MILDEW INFECTION OF THE ONION SEED CROP IN CALIFORNIA FROM 1920 TO 1938, INCLUSIVE

Year	Severity	Average loss in seed crop, per cent
1920	Moderately severe, localized; maximum loss 30 per cent...	2
1921	Similar to 1920	2
1922	No reports available	..
1923	No reports available	..
1924	No reports available	..
1925	Extremely severe on both seed and bulb crop.	60–80
1926	Severe; losses exceeding 50 per cent common in central California and coastal region......	40
1927	Moderately severe on seed crop in localized areas	3
1928	Widely distributed on seed crop	5
1929	Little or none in Sacramento Valley; trace in coastal region	0
1930	Severe in localized areas; maximum loss in any area, 75 per cent	25
1931	Severe and general during March; little spread during April and May but moderately severe in local areas during June	10
1932	Little infection on seed onions, slight on bulb crop	1
1933	Practically none on seed onions	0
1934	Severe and generally distributed on seed onions	50
1935	Similar to 1934	50
1936	Less than in 1934 and 1935	5
1937	Of little consequence in seed-producing districts	2
1938	Severe in localized districts	8

Source of data:

Observations reported in the *Plant Disease Reporter* for certain years with corresponding volume and page citations as follows: 1920, **16**:237; 1921, **22**:354; 1925, **45**:68–69 (supplement); 1926, **54**:279; 1927, **61**:261; and 1928, **68**:59. All other observations are by the authors.

seasons, the disease became epidemic by March but subsequent environmental conditions were such that only relatively slight infection was evident on seedstalks.

SOURCES OF INOCULUM

Mycelium in Bulbs.—There is abundant evidence that infected onion bulbs harbor the mycelium of the organism, as was first demonstrated by Murphy and McKay (*8*). Plants systemically infected with mildew have been found in seed fields in California, and these are probably the initial sources of inoculum. Additional importance has been given to this method of hibernation by Newhall's observation (*10*) that infected Egyptian or topset onions and potato or multiplier onions serve as im-

portant sources of primary downy-mildew inoculum in the important onion-bulb sections of New York state.

Oöspores in Leaves and Seedstalks.—Oöspores are formed in the tissues of infected plants, but reports in the literature differ as to their abundance. Murphy and McKay in an early paper (*8*) reported that oöspores rarely occurred in fields under their observation but in a later paper (*9*) stated that oöspores are sometimes present in abundance. In central California, large numbers have been found in both infected leaves and seedstalks during several seasons. McKay (*5*) reported germination of oöspores five years old and later (*6*) observed stimulation of germination in 0.01 and 0.02 per cent potassium permanganate.

Infected or Contaminated Seed.—Several investigators have reported infection of flower parts of onion by downy mildew, and Cook (*2*) has demonstrated the presence of mycelium within the ovule. He also found a few oöspores in water used to wash a quantity of commercial seed. Stuart and Newhall (*11*) later reported circumstantial evidence of seed transmission, but no conclusive evidence of the commercial importance of seed transmission has yet been presented.

PRACTICAL DIFFICULTIES IN RELATION TO FUNGICIDAL CONTROL

The fungicidal efficiency of certain chemicals and their toxicity to conidia of many species of the Phycomycetes have been well demonstrated. The literature need not be reviewed here. The problem in connection with onion downy mildew has been to discover chemical mixtures which will adhere to the waxy surfaces of the leaves and seedstalks, particularly during rainy weather. Definite progress has been made in securing such mixtures.

Several factors contribute to the actual inefficiency of these fungicidal materials when applied to the bulb crop of the so-called "intermediate" group of onions. It is the intermediate bulb crop that is most frequently attacked by mildew. On the peat lands, especially, the foliage growth is very rank and does not dry off readily following fog and dew, which provides ideal conditions for infection and sporulation. In California, this crop is usually seeded in the nursery in late August or early September, and the seedlings are transplanted to the field in December and January. On the sedimentary soils, plants are usually set on raised beds spaced about 3 feet from center to center, with seedlings 3 inches apart in the row and two rows to the bed. On peat soils the plants are set on level ground and the rows are spaced about 9 to 10 inches apart. Development aboveground is relatively slow during January and Feb-

ruary but increases thereafter with the rise in temperature. Since growth occurs mainly at the center and base of the plant, new portions of the younger growing leaves are continually becoming exposed to infection. Thus, it is necessary to make frequent applications in order to protect this new growth. This practice, in itself, is almost impossible of execution because the soil is frequently too wet to permit machine applications. Furthermore, while the disease is not epidemic every year, it sometimes appears suddenly and spreads rapidly during favorable weather.

Difficulties are also encountered in spraying the seed crop; frequent spraying is necessary to keep the developing seedstalks covered with fungicide because these, too, grow at the base and continually expose new areas of the stem to infection. Seedstalks, being long and slender, can be completely covered with fungicides only with great difficulty.

The late bulb crop which is usually seeded in the field in January is seldom injured by mildew because the plants make most of their development during the time of year when the air is relatively dry.

LEAF PRUNING AS A CHECK TO SPREAD OF MILDEW

In 1934, mildew appeared very early in some of the small onion-breeding increase plots at Davis. In a plot of the variety strain Stockton G36 (fig. 1, *A*) practically all of the leaves were infected by March 21. A few of the seedstalks had emerged through the surrounding sheaths. It had been observed that where leaf growth was extremely luxuriant, especially following warm, wet winters, mildew was usually much more severe than on sparse foliage; probably this is because the foliage did not dry so quickly after a fog, dew, or rain, or because such leaves may be more susceptible to injury.

In an effort to protect the above-mentioned plot as much as possible against seedstalk infection, all the leaves were trimmed off and the seedstalks left exposed as shown in figure 1, *B*. The seedstalks continued to grow, very little infection occurred, and a good crop of seed was harvested (fig. 1, *C*). While no unpruned plants were left for comparison, the removal of the leaves seemed to have very little deleterious effect on the normal development of the seed crop.

RELATIVE SUSCEPTIBILITY OF VARIETIES

Comparisons of susceptibility were made in 1934 on a number of commercial varieties and foreign introductions planted under overhead irrigation. From one to eight small plots of each lot of the garlic or onion types listed in table 2 were planted in a compact block. On March

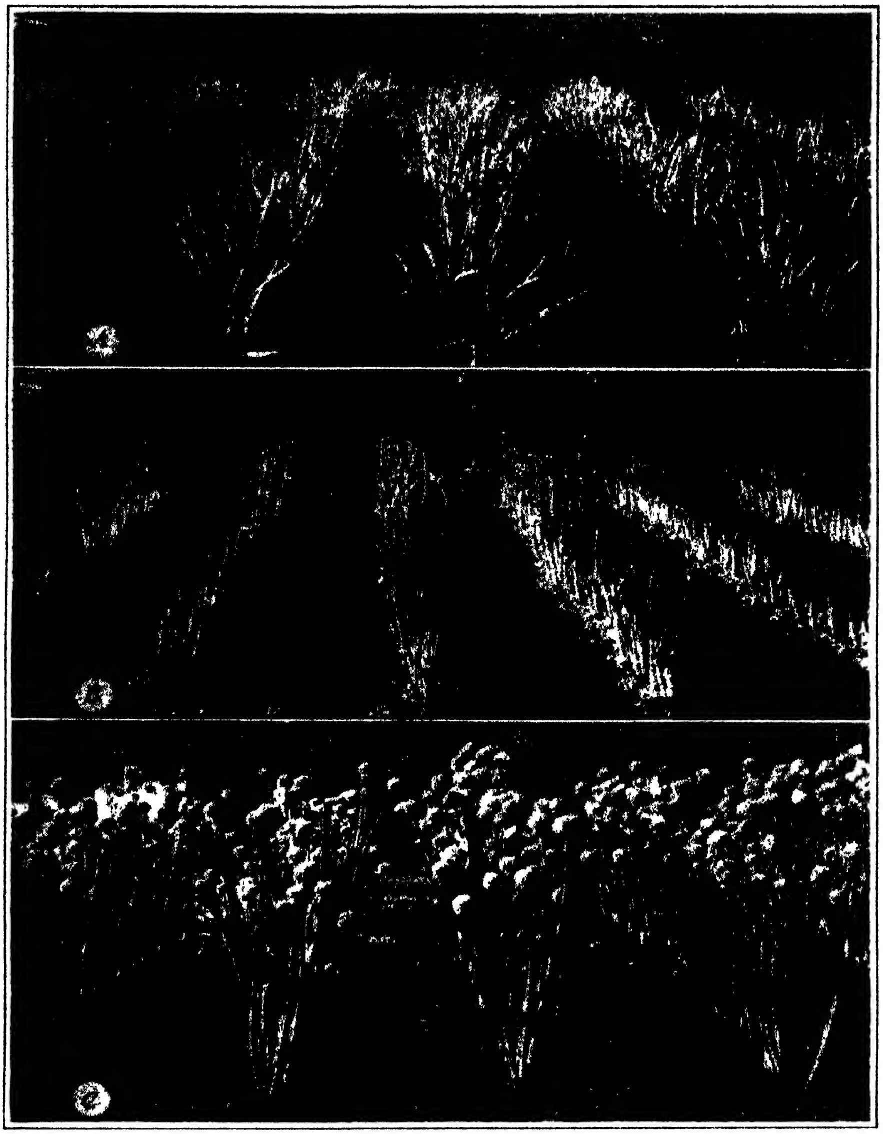

Fig. 1.—*A*, Increase plot of Stockton G36 with luxuriant growth of foliage heavily infected with mildew; photographed March 21, 1934. *B*, The same plot with the mildewed leaves removed to expose the young seed stems; photographed March 22, 1934. *C*, The same plot in full bloom. A normal crop of seed was harvested, indicating that leaf pruning probably did not reduce vigor.

9, and at intervals of a few days thereafter, all plants were sprayed with a suspension of downy-mildew conidia. Humid conditions were maintained by overhead sprinkling. The first sporulation was observed on March 15, and by March 22 infection was abundant and severe on most of the plants. Since in this case the interval (6 days) between inoculation and sporulation was considerably less than that reported by other workers (*2, 13*) under controlled conditions, it appears likely that part of the infection originated from diseased plants previously transplanted into the plot or from wind-borne conidia.

Ratings of the severity of infection on leaves and on seedstalks were made on April 10. The degree of infection was rated from 0 (no infection) to 10 (severe infection), and the results are summarized in table 2. The leaves of all onion varieties showed infection and only commercial Italian Red, Italian Red 13–20–3, and F.P.I. no. 101113 could be considered even moderately resistant. A satisfactory rating of infection on seedstalks could be obtained on only those varieties that produced well-developed seedstalks by April 15, since conditions became unfavorable for uniform infection after that time. Among the varieties forming seed stems early, Italian Red 13–20–3, Early Grano, White Sweet Spanish, and Yellow Strassburg showed the least seedstalk infection; among the most susceptible were Crystal White Wax, Yellow Bermuda, Creole, White Persian, and all of the foreign plant introductions except numbers 101112 and 101113. No infection was observed on garlic.

In the same year an epidemic occurred early in the season on the foliage of the bulb crop of the intermediate varieties such as California Early Red. The leaves of these were badly infected with the exception of Italian Red 13–53, a selection that had been carried along in the breeding plots because of its male sterility. Also the F_1 seedstalks of a cross between Red 21 and 50–6–1, a strain of Stockton Yellow Flat, showed immunity. The foliage and seed stems of all hybrids between *Allium fistulosum* and *Allium cepa* were extremely susceptible (fig. 2).

Another severe downy-mildew epidemic occurred in the breeding plot at Davis in the early months of 1935. Bulbs of certain varieties of the 1934 crop, harvested in July, had been planted in September. The foliage of most plants was badly infected, resulting in premature death of the leaves soon after seedstalk emergence; but the foliage of Italian Red 13–53 again manifested marked resistance. Complete infection was evident on the seedstalks of all varieties and strains except the two Italian Red selections, 13–53 and 13–20–3. Even under the most extreme conditions of infection the seedstalks appeared immune—no lesions were found on them. Many inbred lines and hybrids of susceptible varieties

were killed. In other progenies the primary seedstalks were killed and the secondary stalks were so severely injured that relatively low yields of seed were secured.

TABLE 2

RELATIVE SEVERITY OF DOWNY MILDEW ON THE FOLIAGE AND SEEDSTALKS OF ONION VARIETIES AND GARLIC AT DAVIS IN 1934

Variety or type	Degree of infection*	
	Leaves	Seedstalks
Late garlic	0.0	
Italian Red 13-20-3	6.3	2 5†
Italian Red (commercial)	5 0	
Yellow Strassburg	8.3	2 5
Early Grano	10.0	2.5
White Sweet Spanish	8.3	3 0
F. P. I. 101113	5.0	
F. P. I. 101112	8.3	5 0
Mountain Danvers	10 0	5 0‡
Southport Yellow Globe	7 5	5 0
White Portugal	8.3	5 0
Ohio Yellow Globe	8 3	7.0
Extra Early Red Flat	8 3	6 2
Ebenezer	8 3	6 2
Australian Brown 5-24	10 0	6 2
Yellow Globe Danvers	8 3	7 5
Southport Red Globe	8 3	7 5
Southport White Globe	8 3	7 5
Red Wethersfield	8 3	7 5
Prizetaker	10 0	7 5
Early White Barletta	10 0	7 5
White Persian	7 5	8 3
Creole	10 0	8 3
Yellow Bermuda	10 0	9 2
Crystal White Wax	10 0	10 0
F. P. I. 101171	10 0	10 0
F. P. I. 101224	10 0	10 0
F. P. I. 101460	10 0	10 0
F. P. I. 101461	10 0	10 0
F. P. I. 101499	10 0	10 0
F. P. I. 101515	10 0	10 0

* In the original field data, five degrees of infection were distinguished ranging from 0 (no infection) to 4 (very severe) with plus or minus ratings to identify intermediate groups. To conform to the system of rating used to measure mean injury (table 4) the original ratings were converted to a scale ranging from 0 to 10.

† Although these seedstalks were not examined microscopically, later examination of other seedstalks of this strain showed that similar mildewlike lesions were free from mildew mycelium.

‡ Rating unreliable owing to late formation of seedstalks.

In 1936 several domestic varieties and foreign introductions of *Allium cepa* were grown at Berkeley. White Persian, a thrips-resistant variety recently described by Jones, *et al.* (*3*), was found to be particularly susceptible, many plants being killed by downy mildew before emergence of the seedstalks. Relatively severe infection occurred in leaves and seed-

stalks of Yellow Bermuda, Nebuka, Red Creole, Lord Howe Island, Earliest Express, Giant White Italian Tripoli, Yellow Strassburg, and Southport White Globe. Leaf infection was not noted on Italian Red 13–53. Small mildewlike spots appeared on seedstalks of Italian Red 13–20–3 and were at first thought to indicate downy-mildew infection.

Fig. 2.—Severely infected seedstalks on F_1 plants of Nebuka (*Allium fistulosum* × Australian Brown) in the breeding plot at Davis, California, in March, 1934.

As indicated later, however, the seedstalks of 13–20–3 were immune from infection at Milpitas in 1937 and 1938[6] when 100 per cent infection was noted on many commercial varieties. This apparent discrepancy needs explanation. Small mildewlike lesions frequently occur on seedstalks of 13–53 and 13–20–3 but sporulation has never been observed and microscopic examination of cross sections of such material has never revealed either mycelium or haustoria of the mildew fungus. It is possible that the seedstalk infection charged to 13–20–3 at Berkeley was not actually downy mildew.

[6] Dr. C. E. Yarwood was responsible for the readings made in Berkeley in 1936. He also gave valuable assistance in making the readings at Milpitas in 1937 and 1938.

BREEDING FOR DOWNY-MILDEW RESISTANCE

Three onion strains resistant to downy mildew have been isolated to date. In 1934 the F_1 population (M18) of Red 21 × 50–6–1 produced mildew-free seedstalks. Since Red 21 is susceptible, the resistance of M18 was probably inherited from 50–6–1. The latter strain was an inbred line of Stockton Yellow Flat; and, because seed is no longer available, data on its response to mildew cannot be secured.

The two other resistant strains, numbers 13–53 and 13–20–3, are selections from the variety Italian Red. This variety is of minor importance in California and is seldom grown in other states. Seed is planted in late August, seedlings are transplanted in late November or December, and the plants mature in July in central California. The bulbs are torpedo-shaped, large, red, and mild-flavored. High yields are the rule. The bulbs are poor keepers and for this reason mother bulbs are usually planted in the field in September. Seedstalks of this variety are usually tall and vigorous, producing relatively heavy yields of seed.

Italian Red 13–53.—The Italian Red population from which strain 13–53 was selected was grown at Davis in 1924. In August, a considerable number of desirable bulbs were selected and planted for self-pollination. In April, 1925, the umbels were covered with manila-paper bags when the first flowers opened. The bags were tapped frequently to facilitate self-pollination. These bagged heads were harvested on August 8, and the seed threshed, washed, and dried. From the 5 seed heads of plant 53, 136 bulbils were secured, but no seed. This discovery appeared to have no immediate practical importance, but certainly was of value in a study of sterility. Accordingly, the bulbils were planted and large bulbs were harvested in the summer of 1926. The latter were planted in August, 1926, and another crop of bulbils harvested in July, 1927. In this manner, 13–53 has been asexually propagated since 1925. Bulbil development and type are shown in figures 3 and 4. Interest soon developed in hybrid onions, and 13–53 served as the female parent in a number of crosses (*4*).

Monosmith[7] found that failure of 13–53 to set seed was due to impotent pollen. She found meiosis in the pollen mother cells to be regular but later certain of the tapetal cells degenerated abnormally, with subsequent death of many or all of the microspores. At dehiscence, the pollen-sac contents were cemented together, remaining within the anther.

[7] Monosmith, Helen Ruth. Male sterility in *Allium cepa* L. Unpublished thesis on file at the University of California Library, Berkeley. 1928.

Fig. 3.—*A*, Mature seed heads of Italian Red 13–53 with flower parts removed to show development of bulbils. Although this strain is male-sterile, hybrid seed is readily produced, by using pollen from plants of other varieties. The umbels shown in *B* were bagged with an umbel of an F_1 plant of 13–53 × Red 21. Note the excellent set of seed in the dehisced capsules; also the bulbils in the same head.

Fig. 4.—At the lower right is a bulb of Italian Red 13–53; at the upper right are bulbs of Lord Howe Island, and at the left is a group of F_1 bulbs involving these two varieties. Note the apparent hybrid vigor of the F_1 hybrid.

The strain is completely male-sterile. During the past twelve years the inflorescences of hundreds of plants of 13–53 have been bagged, but selfed seed has never been found. Fortunately, however, 13–53 forms bulbils, insuring continued asexual propagation.

This selection appears to be the best parent isolated to date for breeding resistant onions. It has a high degree of foliage resistance in field plantings, the amount of injury being negligible even under the most severe epidemic conditions. Infection is usually confined to the tips of the leaves and then grows slowly toward the base. Growth of the fungus in the leaf tissues appears to be exceedingly slow.

Italian Red 13–20–3.—This strain also originated in 1924 as plant 20 of commercial Italian Red. Selfed seed of plant 20 was planted in August, 1925, and plant 3 was selected as a superior bulb in July, 1926. Using bulbs of the 13–20–3 line a California seed company increased the seed supply of this strain in 1929 to replace their own stock of Italian Red. Some of the present Italian Red acreage in California is now 13–20–3. It is similar in type to 13–53 but not male-sterile. This line is well colored and of excellent shape but lacks somewhat in vigor because of inbreeding, and the foliage is not as resistant as that of 13–53.

Since 13–53 and 13–20–3 represent bulbs from the same population of Italian Red, it is evident that there existed in this lot of seed the gene or genes for resistance to downy mildew.

Resistance Tests at Milpitas in 1937.—Because downy-mildew infection at Davis varied significantly from year to year, and because the mildew when it did occur existed in epidemic form only early in the season, it was decided to expose the breeding stocks to infection at Milpitas in the Bay Region, where the disease had been epidemic for several successive years and where conditions most years are favorable for the spread of the disease for a longer time than at Davis. Bulbs were planted at Milpitas during September, 1936, and leaf and seedstalk infection recorded from February to July, 1937. To hasten infection, the first five plants of each strain were artificially inoculated on November 20, 1936, with a conidial suspension from heavily mildewed greenhouse-grown plants. On January 7, 1937, only the inoculated plants showed infection, and, therefore, the remaining population was inoculated at this time. Possibly these artificial inoculations were unnecessary, but because a severe test was essential for evaluating resistance an attempt was made to induce a severe and uniform epidemic. Mildew defoliation of known susceptible types was complete by May 18.

On March 25, when the mean number of leaves to the plant was approximately 35, the total number of leaves and of infected leaves was

determined for each lot. The mean percentage of infected leaves is presented in table 3. Between 74 and 84 per cent of the leaves of Red 21, Stockton G36, Lord Howe Island, and Red Rocco were infected, as compared with only 2 per cent of those of 13–53. As in preceding observations, infection of 13–53 was confined to the tips of the leaves, with no serious interference of the normal leaf functions. Although 30 per cent of all the leaves of 13–20–3 were infected, the injury was not particularly severe. Considerable variation was evident in the amount of leaf infection of the various F_1 hybrids involving 13–53. M23, an F_1 of 13–53 $\times$ 13–52–9–6–S_4, manifested only 16 per cent infection, whereas approximately 75 per cent was observed on two F_1 populations of 13–53 $\times$ Crystal White Wax. Percentage of infected leaves, however, is not a particularly good measure of resistance, for it does not give a true picture of amount of injury. Most of the leaves might be infected and still the amount of injury be negligible.

The seedstalks of each plant were carefully examined on June 18 and indexed according to an arbitrary rating from 0 to 10 (table 3). The rating of 0 indicated no mildew lesions visible. Microscopic examination of many small mildewlike lesions on strain 13–53 failed to detect the organism. A rating of 10 indicated the most severe degree of infection, usually so weakening the seedstalk that only a very small quantity of seed was matured. Damage was of strictly minor importance until the stage represented by a rating of 4 was reached. From a breeding standpoint, plants rating 1 or 2 are almost as valuable as those which merit rating of 0, and only those plants manifesting stage 4 or higher are considered as suffering from mildew injury.

The seedstalks of 13–53, 13–20–3, M22, M23, and M24 were immune. Actually, mildew did not cause serious damage to any plants of M16, M4, M5, or M17. The nonuniformity of infection of the F_1 hybrids involving 13–53 may be attributed to the fact that neither 13–53 nor several of the commercial varieties had been inbred before making the respective crosses. The reaction of the various F_1 hybrids seems to indicate that resistance is inherited as a dominant character and that the resistant parent is heterozygous for resistance.

The varieties, Stockton G36, Red 21, Red Rocco, and Lord Howe Island were all badly infected. Among 23 plants of Lord Howe Island, two remained free of seedstalk infection; but it is thought that these are escapes rather than an expression of resistance. In most cases the populations were too small to get a good frequency distribution of the different classes of infection.

TABLE 3

Relative Resistance of Onion Varieties, Strains, and Hybrids at Milpitas, in 1937

Pedigree	Variety or parents	Generations inbred, or hybrid generations	Leaves infected on March 25, per cent	Number of plants	Distribution of plants among the following eleven classes of seedstalk infection on June 18:											
					0	1	2	3	4	5	6	7	8	9	10	Mean
13-53	Italian Red (male-sterile)		2	26	26	0	0	0	0	0	0	0	0	0	0	9.0
13-20-3	Italian Red	7	30	2	2	0	0	0	0	0	0	0	0	0	0	0.0
M22	13-53×Red Rocco	F_1	47	2	2	0	0	0	0	0	0	0	0	0	0	0.0
M23	13-53×13-52-9-6-S	F_1	16	15	15	0	0	0	0	0	0	0	0	0	0	0 0
M24	13-53×13-20-3	F_1	25	32	32	0	0	0	0	0	0	0	0	0	0	0.0
M16	13-53×Lord Howe Island	F_1	52	24	18	2	2	1	1	0	0	0	0	0	0	0.5
M5	13-53×Stockton G36	F_1	76	8	5	0	2	1	0	0	0	0	0	0	0	0.9
M4	13-53×Crystal White Wax	F_1	74	21	9	5	6	1	0	0	0	0	0	0	0	1.0
M4	13-53×Crystal White Wax	F_1	76	18	9	2	2	2	1	2	0	0	0	0	0	1.4
13-52-9-6-S2	Italian Red	5	54	7	4	0	1	0	0	0	0	0	1	0	1	2 9
M17	13-53×Red 21	F_1	53	85	16	20	20	27	2	0	0	0	0	0	0	1.8
M18	50-6-1×Red 21	F_2	58	36	16	4	5	5	3	1	1	0	1	0	0	1.7
MB10	(50-6-1×Red 21)×Red 21	F_1	72	44	4	4	4	6	4	7	9	2	2	1	1	4.2
MB11	(50-6-1×Red 21)×50-6-1	F_1	51	187	1	6	8	32	36	33	24	23	14	9	1	5.0
36-40	Stockton G36	1	83	9	0	0	1	0	3	0	2	0	1	2	0	5.8
21-22-1	Red 21	2	84	40	0	0	1	0	7	11	3	4	9	5	0	6.2
10	Lord Howe Island		75	23	2	0	1	2	0	0	2	4	3	4	5	6.9
70	Red Rocco		74	2	0	0	0	0	0	0	0	0	0	1	1	9.5

Figure 5 shows the high degree of resistance of both seed stems and foliage of 13–53. Figure 6 shows Crystal White Wax with all leaves killed and all seedstalks very severely injured. These two figures show better than any system of noting the high degree of resistance of 13–53 and the extreme susceptibility of Crystal White Wax.

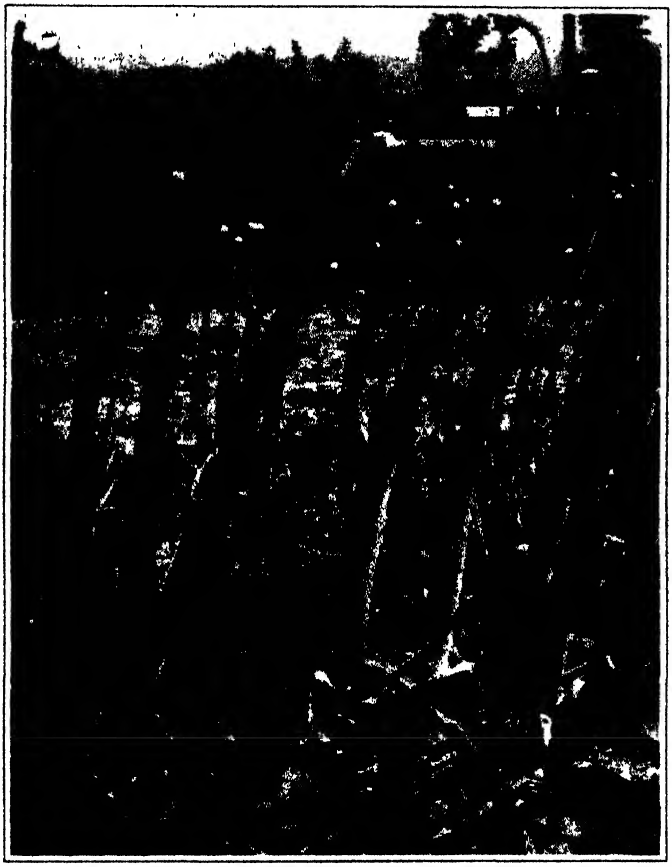

Fig. 5.—Indicating the immunity to downy mildew of seedstalks of Italian Red 13–53 at Milpitas, California, in May, 1937. Note also that the leaves are not severely injured.

Resistance Tests at Milpitas in 1938.—Bulbs for seed production were planted at Milpitas in September, 1937, in the same field where mildew had been so severe that same spring. Many bulbs used had been produced at Milpitas and Sacramento in 1937 and possibly some of these may have also served as sources of infection from perennial mycelium. Weather conditions from December to May were ideal for sporulation and infection. Accordingly, artificial inoculation was unnecessary.

Symptoms were first observed in late December of 1937 and by January 12, 1938, diseased plants were well distributed throughout the plot.

Development of conidia was noted on the foliage on April 6, and these entries appear in table 4 with ratings from 0 to 10, inclusive, as used in other tables. Very striking is the difference in sporulation between Crystal White Wax and the 13–20–3 series as well as that of MB16.

Fig. 6.—Showing extreme susceptibility to mildew of Crystal White Wax at Milpitas, California, in May, 1937. Note the dead leaves and the badly infected seedstalks.

Mother bulbs of 13–53 were not available for inclusion in the seed plot, but seedlings of 13–53 produced by August-planted bulbils were included in the bulb plot at Milpitas. Mildew appeared in this bulb plot in early February. On May 10 and again on June 4 the various populations were rated according to foliage injury. Based on comparative ratings from 0 to 10 on 13–53, foliage injury was only 1 and 2 on the two dates. Lord Howe Island gave readings of 9 to 10 and Crystal White Wax 10 and 10. Strain 13–20–3, however, was only moderately resistant, manifesting stages 4 and 5 injury. Many F_1 populations between susceptible varieties and 13–53 appeared to be about equally resistant, being intermediate between the two parents.

The final readings on injury to the seedstalks, made on July 5, together with the other data are presented in table 4. The sixteen varieties and inbred lines tested may be arranged as follows in the order of decreasing amount of injury: Crystal White Wax, Yellow Bermuda, Yellow Globe Danvers, Early Yellow Globe, Lord Howe Island, Southport

TABLE 4

RELATIVE RESISTANCE OF ONION VARIETIES, STRAINS, AND HYBRIDS AT MILPITAS, IN 1938

Pedigree	Parents or variety	Average degree of sporulation on leaves April 6	Number of plants	Distribution of plants among the following eleven classes of seedstalk infection on July 5:											
				0	1	2	3	4	5	6	7	8	9	10	Mean
13-20-3	Inbred Italian Red	1.5	28	28	0	0	0	0	0	0	0	0	0	0	0.0
	Crystal White Wax	8.6	70	0	0	0	0	0	0	0	0	0	0	70	10.0
	Yellow Bermuda	7.6	50	0	0	0	0	0	0	0	0	0	0	50	10.0
	Yellow Globe Danvers		10	0	0	0	0	0	0	1	0	1	2	6	9.2
	Southport Yellow Globe	6.2	10	0	0	0	0	1	0	2	1	0	0	6	8.3
	Early Yellow Globe	6.6	10	0	0	0	0	0	0	1	0	2	2	5	9.0
	Lord Howe Island	7.8	49	2	1	0	0	2	1	1	1	1	0	40	8.9
	Southport White Globe	6.7	10	0	0	0	0	1	0	1	0	2	0	6	8.6
	White Portugal	7.8	38	0	0	1	0	5	2	2	1	3	3	21	8.1
	Red Rocco	7.9	22	1	0	1	0	3	1	0	0	1	0	15	8.0
	Red 21	2.8	36	0	1	1	1	1	4	5	3	5	8	7	7.3
	Early Grano	4.5	29	2	0	2	0	4	1	2	0	1	0	17	7.4
	Ebenezer	5.6	33	4	0	5	4	8	2	2	1	1	3	3	4.5
	Australian Brown	7.1	35	1	0	9	3	3	0	3	0	2	0	14	6.1
	Sweet Spanish	4.4	37	4	2	6	3	2	2	3	4	1	2	8	5.2
	Stockton G36	6.7	27	10	0	3	1	1	0	2	0	3	0	7	4.4
M4-1-1	13-53×Crystal White Wax F_3	6.4	43	3	2	2	2	3	1	2	1	3	1	23	7.2
M4-1-2	13-53×Crystal White Wax F_3	7.1	36	3	1	0	1	2	2	2	5	3	0	17	7.3
M4-1-3	13-53×Crystal White Wax F_3	7.2	37	4	0	3	0	2	2	1	1	2	1	21	7.4
M4-1-4	13-53×Crystal White Wax F_3	7.1	39	2	0	1	2	5	1	2	0	3	1	22	7.6
M4-1-5	13-53×Crystal White Wax F_3	6.8	27	3	0	3	0	1	3	1	1	5	0	10	6.6
M4-1-6	13-53×Crystal White Wax F_3	7.3	44	4	1	1	0	0	1	2	0	2	5	28	8.2
M4-1-7	13-53×Crystal White Wax F_3	6.2	49	2	2	2	5	2	5	2	1	3	1	24	7.1
M16-2-1	13-53×Lord Howe Island F_3	3.7	90	52	10	16	5	4	2	1	0	0	0	0	1.0
M16-2-2	13-53×Lord Howe Island F_3	3.8	74	41	14	10	3	5	1	0	0	0	0	0	0.9
M16-2-3	13-53×Lord Howe Island F_3	3.7	78	37	7	4	7	3	8	8	3	1	0	0	2.1
M16-2-4	13-53×Lord Howe Island F_3	2.7	10	3	0	2	2	1	1	1	0	0	0	0	2.5
M16-2-5	13-53×Lord Howe Island F_3	4.7	17	10	0	2	1	0	2	1	1	0	0	0	1.8
M28-1-1	13-53×Red Wethersfield F_3	6.0	6	4	0	0	0	1	0	1	0	0	0	0	1.6
M28-1-2	13-53×Red Wethersfield F_3	6.7	26	24	0	2	0	0	0	0	0	0	0	0	0.2
M28-1-3	13-53×Red Wethersfield F_3	5.4	15	11	0	0	1	2	1	0	0	0	0	0	1.1
M28-1-4	13-53×Red Wethersfield F_3	6.8	20	12	4	1	0	1	1	1	0	0	0	0	1.0
M28-1-5	13-53×Red Wethersfield F_3	4.0	90	59	6	10	6	1	1	3	1	2	1	0	1.1
M28-1-6	13-53×Red Wethersfield F_3	4.7	46	28	3	3	4	3	5	0	0	0	0	0	1.3
MB2	(13-53×Crystal White Wax)×Crystal White Wax	7.1	30	0	0	0	0	0	1	0	0	2	0	27	9.7
MB12	(13-53×Early Grano)×Early Grano	3.4	83	31	5	12	10	2	4	6	2	4	2	5	2.8
MB13	(13-53×Red Wethersfield)×Red Wethersfield	5.8	16	10	0	0	2	0	0	0	1	1	1	1	2.5
MB15	(13-53×Lord Howe Island)×Lord Howe Island	4.5	59	22	4	8	5	8	2	3	1	3	1	2	2.6
MB16	(13-53×13-20-3)×13-20-3.	0.4	17	17	0	0	0	0	0	0	0	0	0	0	0.0

White Globe, Southport Yellow Globe, White Portugal, Red Rocco, Early Grano, Red 21, Australian Brown, Sweet Spanish, Ebenezer, Stockton G36, and Italian Red 13–20–3. Again the 13–20–3 family produced mildew-immune seedstalks, and microscopic examination failed to detect mildew mycelium in the yellowed areas of the seedstalks. Although seedstalks of a few plants of Stockton G36 and other varieties were apparently immune, it is more likely that they escaped infection. The seedstalks of Australian Brown, Ebenezer, and Sweet Spanish emerged much later than those of early and intermediate varieties; this may account for the small amount of damage, since even at Milpitas the conditions become increasingly unfavorable for mildew as the season advances.

All bulbs in the various families of the M4 series were white segregates from the F_2 populations. If there is linkage between color and resistance, then it is possible that all the bulbs selected were susceptible, and that the individuals given a rating of 0—and perhaps those of 1 and 2—were escapes.

Bulbs in families M16–2–1 to M16–2–3 were selected at random from the F_2 population. In families M16–2–4 and M16–2–5, flat bulbs were selected. It is almost impossible to classify the present data into well-defined resistant and susceptible classes, since it is not known if the spots found in the seedstalks of plants in classes 1, 2, and possibly 3, are similar to those found on 13–53 which proved to be free from the mildew fungus. Before the definite mode of inheritance can be determined, the seedstalks of borderline plants will probably need to be examined microscopically to determine definitely in what classes they belong. Also, some means must be established to determine the number of escapes in the apparently immune class. The plants in all of the M16 families were extremely prolific, producing many seedstalks per plant and seed heads of large size. The high degree of immunity and vigor exhibited in these F_3 families indicates the early production of an intermediate variety with resistant foliage and immune seedstalks.

The resistance of the six F_3 families of 13–53 × Red Wethersfield (M28 series) was even more striking than the F_3 of the M16 series; but here again the precautions mentioned above will need to be taken in order to determine definitely the mode of inheritance. It is possible that family M28–1–2 is homozygous-resistant since the two plants in class 2 may actually belong to the immunes. The plants were vigorous and produced a high seed yield.

The data for several backcross progenies, indicated as MB strains, are included in table 4. These backcrosses were made in order to develop

mildew-resistant strains typical of the various varieties. These populations, backcrossed to the susceptible parents, had many plants in the immune and highly resistant classes with the exception of MB2, the backcross to Crystal White Wax.

The Italian Red backcross (13–53 × 13–20–3) × 13–20–3 produced mildew-immune seedstalks. This was to be expected, since those of both parents were immune. Although the foliage of 13–20–3 is usually severely damaged under epidemic conditions, relatively little injury is found on the leaves of 13–53. The degree of sporulation recorded in table 4 is 0.4 which suggests that this population when isolated would be subject to rather light spore inoculation. Probably greater foliage resistance can be incorporated by backcrossing to the 13–53 parent, but by doing this pollen sterility will also be increased. Some one of the progenies from this cross may be the foundation stock for a highly resistant variety which should be adapted to about the same range of conditions as the present Italian Red variety—chiefly those of central California.

SUMMARY

Adequate control measures are still lacking for onion downy mildew, and because the disease frequently appears suddenly in epidemic proportions, heavy losses are often incurred by the bulb and seed grower.

Actual losses incurred by seed growers in California, vary from 0 to 80 per cent, with weather conditions the main conditioning factor. During the past nineteen years, the annual reduction in seed yield has been 10 per cent or higher during six seasons, with a maximum between 60 and 80 per cent in 1925, and several annual losses exceeding 40 per cent.

Certain practical difficulties exist in fungicidal control, making especially desirable the development of resistant varieties.

Three sources of resistance have been found. The most promising is strain 13–53, a male-sterile selection from the Italian Red variety. The seedstalks of this strain are immune and the foliage is highly resistant.

Another strain of Italian Red, designated as 13–20–3, likewise manifests seedstalk immunity but the foliage is only slightly resistant. This latter strain, however, is superior to 13–53 in type.

Seedstalk immunity was also found in the year 1934 in an F_1 hybrid between Red 21 and two inbred lines of Stockton Yellow Flat, namely, 50–6 and 50–6–1.

Measured evidence of varietal and hybrid resistance is indicated in the tables and discussed in the text. Certain F_3 and backcross populations involving 13–53 as the resistant parent are particularly promising.

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A GENETIC ANALYSIS OF RED SEED-COAT COLOR IN PHASEOLUS VULGARIS

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A GENETIC ANALYSIS OF RED SEED-COAT COLOR IN PHASEOLUS VULGARIS[1,2,3]

FRANCIS L. SMITH[4]

INTRODUCTION

SEVERAL VARIETIES of red beans are grown commercially in California. The market grades of these beans are determined largely by variations of the red color. The red changes to brownish red and brown after a year or two of storage. The occurrence of brown beans in these red varieties is considered by the trade to indicate old beans. Some varieties, especially Red Kidney, are easily discolored by the sun during the harvest so that occasionally newly threshed beans appear to be a year old.

The present study is a genetic analysis of red seed-coat color in the common bean (*Phaseolus vulgaris* L.) preliminary to a breeding program that might result in the introduction of factors that would stabilize the color of the Red Kidney variety. Commercial conditions are adverse to the introduction of varieties with new colors. The breeding problem, then, resolves itself into making more fast the red color without altering it. The ideal may be visualized as a color between the normal red and a darker red, and it was hoped that such an intermediate type could be developed. Crosses were made between red beans of several varieties. This paper reports the results obtained from these experiments.

REVIEW OF LITERATURE

The common bean is world-wide in distribution and is represented by hundreds of horticultural varieties with scores of seed-coat colors and a number of patterns of distribution of color. The species hybridizes easily. Therefore there is little wonder that the literature on the genetics of this species is voluminous and polylingual. Since different workers used different varieties and described the colors by various standards there is little wonder that the results, too, are variable and often apparently contradictory. There is no standard usage of symbols for the genes which have been analyzed; the same symbol has been used to mean a number of different characters. Beans were used by a number of the

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early hybridists including Mendel (*15*).[5] No attempt will be made here to unravel all the complications and disagreements extant in the literature. This task was undertaken by Kooiman (*7*) in his monograph on the genetics of the genus *Phaseolus*. Reference will be made, however, to earlier workers on those genes encountered in the present studies.

White Color.—Tschermak (*26–29*) was first to report on the white character. In crosses between colored and white beans he assumed a basic factor necessary for color. Later he (*30*) proposed the symbol *A* to represent the presence or absence of this factor. Shull (*22*) used the symbol *P* for yellow or brown pigment and *p* for white; and Emerson (*2, 3*) used the same symbols to represent presence or absence of pigment. In his monograph, Kooiman (*7*) used the *A* symbol to represent the presence of the primary color gene; later workers (*9, 17*) have resorted to the use of *P*. Since the symbol has priority rights this gene will be referred to as *P* in this paper. The conception of *P* is that of a fundamental color gene which of itself gives no color. Thus two types of white beans are possible: *p* whites lacking the fundamental color factor and *P* whites which lack any complementary color genes. This will explain the results of Shaw and Norton (*20*) who obtained colored F_1 plants by crossing two white varieties. Lamprecht (*12*) has obtained *P* white experimentally. Most white varieties, however, are *p* white.

Mottling.—The early workers were greatly concerned with the mottling character. From their results it soon became apparent that there were at least two genetic types of mottling—constant and inconstant.

Some bean varieties are mottled and this is a true breeding character. Tschermak (*28*) showed that mottling was a simple dominant in crosses between mottled and self-colored varieties. He considered the mottling distinct from the color genes. Shull (*22*) designated the symbol *M* for mottled beans and *m* for self-colored. This type of mottling has been studied by a number of workers. Another type of mottling which is similar in breeding behavior was reported by Tjebbes and Kooiman (*25*). The striping factor found in Cranberry beans they thought restricted the expression of the red color to stripes. It was given the symbol *S*. In a later paper Tjebbes (*24*) reported strong linkage between *S*, *B*, and *R*, the latter two being genes for seed-coat color.

Tschermak (*28*), Emerson (*2*), Shull (*21*) and numerous other workers found another type of mottling which was somewhat baffling. Its general characteristics may be seen by the breeding behavior of some crosses. Self-colored × self-colored gave mottled F_1; and F_2 ratio was 1 mottled : 1 self-colored. Self-colored × some white varieties gave mot-

[5] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

tled F_1; and in F_2 the ratios were 3 mottled: 3 self-colored: 2 white. Emerson (*2*) called this type of mottling *X*-mottled in contrast to the true-breeding *M* type. Later he (*3*) proposed two closely linked genes *Y* and *Z* as being responsible for both types of mottling. In the mottled varieties both genes were present as dominants *P YZ*. Self-colored races, each with one dominant and one recessive, when crossed would give the inconstant mottled type. For instance, *P yZ* (self-colored) $\times$ *P Yz* (self-colored) would give a mottled F_1, namely $\frac{P\ yZ}{P\ Yz}$. The F_2 from such a cross would segregate into $1\ \frac{P\ yZ}{P\ yZ}$ (self-colored) : $2\ \frac{P\ Yz}{P\ yZ}$ (mottled) : $1\ \frac{P\ Yz}{P\ Yz}$ (self-colored). The genotype *P yz*, he thought, carries no mottling factors. White beans could carry any combination of mottling factors in a latent condition. Since the *yZ* and *Yz* were completely linked, the breeding behavior is the same as expected for a single heterozygous gene. No crossing-over was ever observed between these two hypothetical genes, so their existence could not be proved. Kooiman (*6*) offered a more likely theory of inconstant mottling. A bean with a heterozygous color gene *B* is mottled. When homozygous for *B* the color is darker, and when homozygous for *b* it is lighter. To conserve space homozygous genes will here be represented by a single symbol and heterozygous by the symbols for the dominant and the recessive allelomorphs. The reaction of the *B* gene can be shown in a single case taken from Kooiman's monograph (*7*): *P B C* = coffee brown, *P Bb C* = coffee brown mottled, *P b C* = sallow yellow.

Kristofferson (*8*) used the symbol *K* to represent the same thing: *P K* = black; *P Kk* = black mottled; *P k* = steel gray. This type of mottling has been worked out in great detail by Lamprecht (*9–12*). The color gene which causes mottling when heterozygous he called *C*. It also acts as a modifier in the presence of other color genes. Its action is illustrated in the following zygotic genotypes where mottling is indicated by a slant-line fraction, with the darker color as the numerator and the lighter color as the denominator: *P C J G B* = argus brown; *P Cc J G B* = argus brown/buckthorn brown; and *P c J G B* = buckthorn brown. Likewise, *P C J* = chamois; *P Cc J* = chamois/raw-silk yellow; and *P c J* = raw-silk yellow. And finally, *P C* = sulfur white; *P Cc* = sulfur white/white; and *P c* = white.

In a later paper Lamprecht (*14*) presented data from a cross between Canadian Express and de la Chine. The color of the former was dark plum violet to Bordeaux red; the latter was sulfur white, which was

shown in previous experiments to be *P C j g b v*. The F_1 was weakly mottled, plum violet/chamois. This mottling could not be due to *Cc* because *P C j* is sulfur white and *P c j* is white. He supposed that the heterozygous gene pair *R r* was the cause of mottling. The color reactions observed in F_2 and F_3 were: *P C J R,* dark plum violet; *P C J Rr,* dark plum violet/chamois; *P C J r,* chamois; *P C j R,* light lilac; *P C j Rr,* light lilac/sulfur white; and *P C j r,* sulfur white.

Red Color.—Shaw and Norton (*20*) first called attention to red color inheritance in beans. They recognized two color series, the yellow-black and the red caused by anthocyanins which they represented by *M* and *M′* respectively. Further red modifiers were postulated: *E* for purplish red as in the variety Mohawk, and *D* for light red as in Red Valentine. The supposition of the *M* and *M′* factors seems superfluous in the light of more recent work. Tjebbes and Kooiman (*25*) used three genes to account for the color in Cranberry beans, namely, *R, Bl,* and *Z*. Their interactions were represented as follows: *Rr,* pale red; *R,* red; *R, Z,* brownish black; *Rr Bl,* violet; *R bl,* purple; *Rr Bl Z,* bluish gray; *R Bl Z,* black. The color due to *r* only was not indicated. Tjebbes (*24*) described wine red as *R c* and Burgundy red as *R C*. The genes *R* and *S* (*S* for striping) were linked with about 1 per cent crossing-over. Reference has already been made to Lamprecht's (*14*) red gene and its phenotypic expression. In some of the crosses reported in the present paper there is segregation for a gene which is similar to Lamprecht's (*14*) *R* because beans heterozygous for this red gene are mottled. Therefore the symbol *R* is used, assuming it is the same gene as Lamprecht's *R*.

Gloyer (*5*) reported progeny tests from a cross, White Kidney × Red Kidney. He made no attempt to analyze the genetics of color, but merely presented his data. Since his data support those obtained in this work they will be summarized later. The red color of Red Kidney behaved as a recessive, the dominant allelomorph being buff. This gene will hereafter be designated as *Rk*.

Eyed Beans.—Emerson (*3*) studied the heredity of partial color in beans. Self-colored × eyed, gave self-colored in the F_1; and in the F_2 they segregated into 3 self-colored : 1 eyed. White × eyed gave self-colored in the F_1, and in the F_2 they segregated into 9 self-colored : 3 eyed : 4 white. He postulated two genes, *P*, the primary pigmentation factor, and *T* a gene which restricted color to the area about the hilum. The interaction was 9 *P T,* self-colored : 3 *P t,* eyed : 4 *p,* white. In addition, he proposed the symbol *E* for self-colored, and *e* for eye pattern. Tschermak (*30*) used the symbol *Z* to represent this pair of genes. Surface (*23*) grew progenies from natural hybrids between New Improved Yellow

Eye (large-eye pattern) and Old Fashioned Yellow Eye (small-eye pattern). The F_1 was piebald, with the color irregularly dispersed over most of the seed. In F_2 he observed 146 piebald : 50 large eye : 70 small eye. He thought the low number in the large-eye class was due to linkage of the pattern factor and a lethal; but his hypothesis was not proved. If the data are fitted to a 2 :1 :1 ratio by χ^2 goodness of fit test, the probability value is .12. The secondary assumption therefore seems ungrounded. The results of Shaw and Norton (*20*) were explained by Emerson's (*3*) *P T* hypothesis. Sax (*19*) believed the eye pattern was due to a double recessive condition for *t* and *e*, because white × eyed gave an F_2 which fitted the ratio of 45 colored : 3 eyed : 16 white better than it did a 9 :3 :4 ratio. Miyaki, *et al.*, (*16*) crossed two partially colored types, saddle × bald, and in F_2 obtained a ratio of 12 bald : 3 saddle : 1 eyed. Lamprecht (*13*) found five genes responsible for twenty-two partial color patterns. These genes were independent of four color genes. In pattern his partial-colored types varied from a dot on either end of the hilum scar to almost complete self color. The dot type was due to the recessive condition of the *bip* (bipunctata) gene; the dominant *Bip* had a "virgarcus" pattern. In the experiments reported in this paper there is but one eye pattern which is similar to Lamprecht's "virgargus" (plate 1, figs. 34, 35). It will be represented by the symbol *E* (self-colored) and *e* (eyed) following Emerson's (*3*) nomenclature.

Colored Hilum Ring.—According to Lamprecht (*12*) three color genes also color the hilum ring in the presence of the ground factor *P*. These are *B*, *J*, and *G*. Prakken (*17*) also noted colored hilum rings with his genes, *S*, *C*, and *V* (probably identical to Lamprecht's *B*). In the studies reported here segregation for hilum ring was found only in some crosses involving the variety Mexican Red which has a black hilum ring.

MATERIALS AND METHODS

The crosses made to provide material for genetic analysis involved varieties of red beans, mottled beans that were predominantly red, white beans, and derivatives from these crosses. In the hybrids it was soon found that some standard of color must be employed to designate the different tints and shades. Ridgway's (*18*) *Color Standards and Color Nomenclature* was used. In order to save time in matching, each time a new color type appeared it was matched with the color book and a specimen sample placed in a Riker mount and labeled with the color name; the beans in each progeny were then matched with the type specimens. These standards represented modal classes, allowing for slight variations. The distinguishable colors are more numerous than the pheno-

types so that the grouping of several closely related colors is necessary to avoid confusion in studying the actual phenotypes. This becomes apparent in F_3 progeny tests made from beans which were classified for color in F_2.

The varieties used in these crosses together with the author's accession numbers were:

Red Kidney 4370 (plate 1, fig. 1)
Red Kidney 4395
Geneva Red Kidney 4387
Nagazura 4390 (plate 1, fig. 2)
Speckled Kidney 50(51)30 (plate 1, fig. 5)
Long Roman 4521 (plate 1, fig. 3)
Red Eye 4387 (plate 1, fig. 34)
White Kidney 4516 (plate 1, fig. 33)
White Kidney of different genotypes derived from F_3 Red Kidney × White Kidney
Dark Red Kidney (65)31 (plate 1, fig. 4)
China Red 4414 (plate 1, fig. 6)
Mexican Red 4437 (plate 1, fig. 8)
Buff (plate 1, fig. 7) derived from true-breeding F_3 extracts of Nagazura × Red Kidney

RESULTS

In this discussion of results the colors of mottled beans are written as a fraction as explained in the section "Review of Literature"; this usage has already been accepted in the literature as indicating mottling. In the tables the zygotic genotypes are represented as follows: heterozygous genes are shown as a fraction, the dominant allelomorph as the numerator and the recessive as the denominator; homozygous genes, either dominant or recessive, are represented by a single symbol. This method makes it easier for the reader to see which genes are segregating. The χ^2 method was used as a measure of goodness of fit. The probability values (P) shown in the tables were taken from Fisher's table for the χ^2 values (*4*). Interpolations of probability were made for χ^2 values which were intermediate between any two values given in the table.

The genetic analysis of the crosses made are discussed in the following paragraphs. Each cross is treated separately and where possible the genotypes of the parents are indicated by symbols in the topic heading.

NAGAZURA × RED KIDNEY

(Formula: *P M Rk bl* × *P m rk Bl*)

Nagazura is a red/buff mottled bean (plate 1, fig. 2). The F_1 was purple/buff (plate 1, figs. 9, 10, 11). It is assumed, therefore, that the Red Kidney (plate 1, fig. 1) carries a gene which changes the red in a mottled bean to purple. This gene is similar in action to the *Bl* described by Tjebbes and Kooiman (*25*) and will therefore be designated by this symbol. In the presence of the recessive *bl*, mottled beans are red-mottled. Since both these varieties are colored, they both carry *P*. Nagazura

carries *M*, the mottling gene. In the F_2 only two self-colored classes were obtained, buff (plate 1, fig. 7) and testaceous, like the Red Kidney (plate 1, fig. 1). Since other red colors will be encountered later, this shade of red will be known as testaceous. This gene pair is represented by *Rk* (buff) *rk* (testaceous). The F_2 should segregate for three genes: *M, Rk,* and *Bl.* Since the red parent contributed *Bl* the buff phenotypes may be *P m Rk Bl* or *P m Rk bl* and the testaceous, *P m rk Bl* or *P m rk bl.* In other words, the presence of *Bl* cannot be distinguished in the self-colored segregates. In the mottled beans four classes can be distinguished as follows: *P M Rk Bl* purple/buff, *P M rk Bl* purple/testaceous, *P M Rk bl* red/buff, and *P M rk bl* red/testaceous. Thus, the *Rk* gene can be distinguished in both mottled and self-colored beans, *m Rk* being buff self-colored, *M Rk* mottled on buff background, *m rk* testaceous self-colored, and *M rk* mottled on testaceous background. The *Bl bl* pair can be distinguished only in the mottled types, *M Bl* being purple-mottled and *M bl* red-mottled. The expected ratio in F_2 should be 27 *P M Rk Bl* purple/buff: 9 *P M rk Bl* purple/testaceous: 9 *P M Rk bl* red/buff: 3 *P M rk bl* red/testaceous: 12 *P m Rk* buff: 4 *P m rk* testaceous. F_3 progeny tests were made of a few F_2 phenotypes. The results of F_2 and F_3 from this cross are presented in table 1.

If the assumptions in respect to the genotypes are correct, the purple/buff (*P M Rk Bl*) should segregate for all three, any two, any one, or none of the genes *M, Rk, Bl.* In the progenies tested one segregated for *M, Rk, Bl;* one for *M, Bl;* one for *M Rk;* and one for *Bl.*

The purple/testaceous (*P M rk Bl*) should segregate for either one or both of the genes *M* and *Bl.* No purple/buff, red/buff, or buff segregates are expected because all F_2 beans of this phenotype are homozygous for *rk.* Only one progeny test was made. It segregated for *M* and *Bl.* The red/buff (*P M Rk bl*) should segregate for only two genes at most, *M* and *Rk.* No purple mottled are expected in any of the progeny because they all carry *bl.* One of those tested segregated for *M* and *Rk* and another bred true. The buff phenotype (*P m Rk*) should segregate for *Rk* or breed true. Four progeny tests were made; three segregated for *Rk,* the other bred true. The testaceous phenotypes (*P m rk*) should all breed true, and four progeny tests made of this phenotype did so.

SPECKLED KIDNEY × RED KIDNEY

(Formula: *P M Rk bl* × *P m rk Bl*)

The maternal parent of this cross (plate 1, fig. 5) is red/buff. The F_1 was purple/buff (plate 1, fig. 9). The F_2 should segregate 27 *P M Rk Bl* purple/buff: 9 *P M rk Bl* purple/testaceous: 9 *P M Rk bl* red/buff: 3

TABLE 1
NAGAZURA × RED KIDNEY, CROSS NO. 30.017
(Formula: $P\ M\ Rk\ bl \times P\ m\ rk\ Bl$)

Parental colors	Parental genotypes	Expected ratios in progenies	Progenies tested	Segregants: Purple/buff ($P\ M\ Rk\ Bl$)	Segregants: Purple/testaceous ($P\ M\ rk\ Bl$)	Segregants: Red/buff ($P\ M\ Rk\ bl$)	Segregants: Red/testaceous ($P\ M\ rk\ bl$)	Segregants: Buff ($P\ m\ Rk$)	Segregants: Testaceous ($P\ m\ rk$)	Segregants: Total	Probability values
	F₁ generation			F₂ generation							
Purple/buff	$P\frac{M}{m}\frac{Rk}{rk}\frac{Bl}{bl}$	27·9·9:3 12·4	4	71	33	40	12	39	10	205	0.12
	F₂ generation			F₃ generation							
Purple/buff	$P\frac{M}{m}\frac{Rk}{rk}\frac{Bl}{bl}$	27:9:9.3:12 4	1	6	1	2	4	3	0	16	0.01
	$P\frac{M}{m}Rk\frac{Bl}{bl}$	9:0:3·0·4 0	1	16		1		2		19	0.05
	$P\ M\ Rk\frac{Bl}{bl}$	3·0 1·0 0 0	1	8		4				12	0 80
	$P\frac{M}{m}\frac{Rk}{rk}Bl$	9 0 3 0 3 1	1	5		1		0	2	8	0 10
Purple/testaceous	$P\frac{M}{m}rk\frac{Bl}{bl}$	0 9 0.3.0.4	1		7		4	.	4	15	0.86
Red/buff	$P\frac{M}{m}Rk\ bl$	0 0 3 0 1 0	1			9	.	2		11	0.87
	$P\ M\ Rk\ bl$	0 0.all	1			9	.	.	.	9	. . .
Buff	$P\ m\frac{Rk}{rk}$	0 0·0:0.3:1	3				.	64	10	74	0.02
	$P\ m\ Rk$	0:0.0:0:all	1				.	24		24	. . . .
Testaceous	$P\ m\ rk$	0:0:0:0:0:all	4			. .	.	.	95	95	. . . .

P M rk bl red/testaceous : 12 *P m Rk* buff : 4 *P m rk* testaceous, as did the last cross. Results are shown in table 2. The F_2 with 275 plants gave a probability value of 0.05 fitted to such a ratio. In F_3 the same results should be expected as reported in table 1. Five purple/buff F_3 progenies segregated for *M, Rk,* and *Bl;* one for *M* and *Rk;* three for *M* and *Bl;* three for *Rk* and *Bl;* and three for *Rk.* The purple/testaceous had one progeny segregating for *M* and *Bl;* two for *M;* and three for *Bl.* Two bred true. Three red/buff segregated for *M* and *Rk,* one for *Rk,* and two bred true. Four red/testaceous progenies segregated for *M.* In one of these progenies, there unexpectedly appeared two purple/testaceous plants; these were probably due to natural hybridization with a purple-mottled bean. Three red/testaceous F_2 plants bred true in F_3. Five progenies from buff segregated for *Rk* and six bred true. Eight testaceous progenies bred true as expected.

RED KIDNEY × LONG ROMAN

(Formula: *P m rk Bl* × *P M Rk bl*)

There are two crosses grouped together in table 3. Long Roman (plate 1, fig. 3) is red/buff. The F_1 was purple/buff, so similar results are expected in these crosses as in the preceding ones. The probability value for χ^2 is very small. For this reason the calculated numbers are here given for each color group. The major discrepancy is the low number of purple/testaceous plants and the high number of red/testaceous. The self-colored testaceous class is also low. Is this discrepancy due to some disturbing genetic conditions or could it be due to errors in classification? The three segregating genes are *M, Rk,* and *Bl.* There were 256 mottled (*M*) and 78 self-colored (*m*). This fits a 3:1 ratio with a probability value of 0.49. The total number of beans with *Rk* were 262 and with *rk,* 72. This fits a 3:1 ratio with a probability value of 0.16. Only mottled beans show reaction for the *Bl* gene. There were 168 *Bl* and 88 *bl.* For a 3:1 ratio, 192:64 is expected. The probability value is 0.05. Thus it appears that each gene taken separately fits the expected ratios fairly well.

Linkage between *M* and *Bl* cannot be measured because *Bl* is not apparent in self-colored (*m*) beans. Segregation for *M* and *Rk* was: 196 *M Rk,* 60 *M rk,* 66 *m Rk,* and 12 *m rk.* Fitted to a 9:3:3:1 ratio the expected numbers are 187.87: 62.63: 62.63: 20.87, respectively, with a probability value of 0.22, indicating no linkage. Segregation for *Rk* and *Bl* can be studied only in the mottled beans. The segregation of *Rk* and *Bl* was: 144 *Rk Bl,* 52 *Rk bl,* 24 *rk Bl,* and 36 *rk bl.* For a 9:3:3:1 ratio the calculated numbers for these classes are 144: 48: 48:16. Thus the

TABLE 2

SPECKLED KIDNEY × RED KIDNEY, CROSSES NOS. 30.020 AND 30.021

(Formula: *P M Rk bl* × *P m rk Bl*)*

Parental colors	Parental genotypes	Expected ratios in progenies	Progenies tested	Segregants							Probability values
				Purple/buff (M Rk Bl)	Purple/testaceous (M rk Bl)	Red/buff (M Rk bl)	Red/testaceous (M rk bl)	Buff (m Rk)	Testaceous (m rk)	Total	
	F_1 generation			F_2 generation							
Purple, buff Cross no 30 020	$\frac{M}{m}\frac{Rk}{rk}\frac{Bl}{bl}$	27.9.9.3.12·4	1	27	11	11	1	22	5	77	0.23
Cross no. 30 021	$\frac{M}{m}\frac{Rk}{rk}\frac{Bl}{bl}$	27.9.9 3:12:4	4	88	17	37	6	37	13	198	0.13
Total	$\frac{M}{m}\frac{Rk}{rk}\frac{Bl}{bl}$	27.9·9.3.12 4	5	115	28	48	7	59	18	275	0.05
	F_2 generation			F_3 generation							
Purple/buff	$\frac{M}{m}\frac{Rk}{rk}\frac{Bl}{bl}$	27:9.9 3.12.4	5	78	21	36	11	25	15	186	0.11
	$\frac{M}{m}\frac{Rk}{rk}Bl$	9·3 0:0.3.1	1	16	10		.	9	1	36	0.28
	$\frac{M}{m}Rk\frac{Bl}{bl}$	9 0 3 0 4 0	3	71		30		28	..	129	0.38
	$M\frac{Rk}{rk}\frac{Bl}{bl}$	9 3.3 1:0 0	3	69	23	25	3	..	..	120	0.25
	$M\frac{Rk}{rk}Bl$	3 1 0·0:0·0	3	61	23			..		84	0.63

Purple/testaceous........	$\frac{M}{m}$ rk $\frac{Bl}{bl}$	0 9:0 3 0 4	1		13		2		3	18	0 40
	$\frac{M}{m}$ rk Bl	0.3 0.0 0 1	2		43				25	68	0 02
	M rk $\frac{Bl}{bl}$	0:3:0.1:0.0	3		56		20	.		76	0.79
	M rk Bl	0·all	2		61	..	.			61	....
Red/buff..	$\frac{M}{m}$ $\frac{Rk}{rk}$ bl	0·0·9 3 3 1	3			64	16	26	5	111	0.41
	M $\frac{Rk}{rk}$ bl	0 0.3 1 0 0	5	.	.	104	29	..	..	133	0.70
	$\frac{M}{m}$ Rk bl	0·0·3 0 1 0	1	.	..	20	..	4	..	24	0 36
	M Rk bl	0.0 all	2	..	..	41	..	..	..	41	....
Red/testaceous . . .	$\frac{M}{m}$ rk bl	0·0 0 3·0 1	3	..	.	..	82	..	23	105	0 47
	$\frac{M}{m}$ rk bl	0 0 0.3·0.1	1	..	2†	..	12	..	4	18	...
	M rk bl	0·0·0.all	3	..	..	..	84	..	.	84	...
Buff	m $\frac{Rk}{rk}$	0·0 0·0.3 1	5	.	..	..	..	106	30	136	0 44
	m Rk	0·0.0:0.all	6		..	.	..	181	.	181	
Testaceous	m rk	0.0:0:0:0·all	8		.	.	..	..	233	233	. .

* All genotypes in the table are homozygous for P.
† Probably field hybrids, with pollen from a purple-mottled bean.

TABLE 3

RED KIDNEY × LONG ROMAN, CROSSES NOS. 33.048 AND 33.052

(Formula: $P\ m\ rk\ Bl \times P\ M\ Rk\ bl$)*

Parental colors	Parental genotypes	Expected ratios in progenies	Progenies tested	Segregants							Probability values
				Purple/buff ($M\ Rk\ Bl$)	Purple/testaceous ($M\ rk\ Bl$)	Red/buff ($M\ Rk\ bl$)	Red/testaceous ($M\ rk\ bl$)	Buff ($m\ Rk$)	Testaceous ($m\ rk$)	Total	
F₁ generation				F₂ generation							
Purple/buff: Cross no. 33.048	$\frac{M}{m}\ \frac{Rk}{rk}\ \frac{Bl}{bl}$	27:9:9:3:12:4	5	58	11	24	17	27	3	140	Low†
Cross no. 33.052	$\frac{M}{m}\ \frac{Rk}{rk}\ \frac{Bl}{bl}$	27:9:9:3:12:4	5	86	13	28	19	39	9	194	Low
Total	$\frac{M}{m}\ \frac{Rk}{rk}\ \frac{Bl}{bl}$	27:9:9:3:12:4	10	144	24	52	36	66	12	334	Low
F₂ generation				F₃ generation							
Purple/buff	$\frac{M}{m}\ \frac{Rk}{rk}\ \frac{Bl}{bl}$	27:9:9:3:12:4	1	17	8	4	1	4	3	37	0.60

* All genotypes in the table are homozygous for P.

† The word "low" is used in those cases where the probability value is less than 0.01.

purple/testaceous (*rk Bl*) class is too small and the red/testaceous (*rk bl*) class is too large for a good fit. The probability value is very low when these data are fitted to such a ratio. Since the *Rk* gene came from one parent, and the *Bl* from the other, the double recessive class *rk bl* should be low if linkage were the cause of the poor fit. As a matter of fact the reason for the poor fit is that this class is too large, which leaves no explanation for the discrepancy except a failure to accurately distinguish between purple/testaceous and red/testaceous color classes.

A single F_2 purple/buff was tested in F_3. Fitted to the expected ratio for three independently segregating genes these data showed no discrepancy as observed in the F_2, the probability value being 0.60.

RED EYE × RED KIDNEY

(Formula: *P e rk* × *P E rk*)

Red Kidney is testaceous self-colored (plate 1, fig. 1); Red Eye is a white kidney bean with a red eye pattern like Lamprecht's (*13*) "virgarcus" (plate 1, figs. 34, 35).

Now if the red color is genetically the same in both varieties, we should expect a monohybrid segregation for eye pattern, *e*. The F_1 was testaceous self-colored. The F_2 and F_3 ratios are shown in table 4. The probability value for F_2 data fitted to a 3:1 ratio was 0.33 and for nine segregating families in F_3 it was 0.84. Twelve F_3 families from testaceous F_2 were tested; 9 segregated testaceous eye and 3 bred true. The probability value is 0.55 when these data are fitted to the expected 2:1 ratio.

The red color in Red Eye is therefore genetically the same as in Red Kidney. These varieties differ only in the gene *e* for eye pattern. As pointed out by Lamprecht (*13*) this type of pattern may in fact be due to a dominant *Bip* ("virgarcus") gene, the recessive *bip* (bipunctata) not being present. The genotype for Red Kidney then is *P E Bip rk* and for Red Eye *P e Bip rk*.

BUFF × RED EYE, AND RECIPROCAL

(Formulas: *P E Rk* × *P e rk* and *P e rk* × *P E Rk*)

The buff beans used in these reciprocal crosses were true-breeding F_3 extracts from the cross reported in table 1 and were therefore of the genetic constitution *P m Rk*. If the assumptions are true for the cross reported in table 4, the results can be predicted for these The F_1 should be buff self-colored and the F_2 should segregate into 9 *P E Rk* buff self-colored : 3 *P e Rk* buff eye : 3 *P E rk* testaceous self-colored : 1 *P e rk* testaceous eye. The results are shown in table 5. They are in conformity with expectations. These results prove that the red (testaceous) found

TABLE 4

Red Eye × Red Kidney, Cross No. 30.018

(Formula: $P\ e\ rk \times P\ E\ rk$)

Parental colors	Parental genotypes	Expected ratios in progenies	Progenies tested	Segregants			Probability values
				Self-colored testaceous ($P\ E\ rk$)	Red eye, testaceous ($P\ e\ rk$)	Total	
F_1 generation				F_2 generation			
Self-colored testaceous	$P \frac{E}{e} rk$	3·1	3	221	64	285	0.33
F_2 generation				F_3 generation			
Self-colored testaceous* .	$P \frac{E}{e} rk$	3 1	9	192	62	254	0 84
	$P\ E\ rk$	all.0	3	92	...	92	....
Red eye (testaceous)	$P\ e\ rk$	0·all	6	.	125	125	..

* There were 9 self-colored families which segregated eyed progenies, and 3 which bred true. The theoretical ratio is 2:1. The probability value when fitted to the theoretical ratio is 0.55.

TABLE 5

BUFF × RED EYE (CROSS NO 34.162) AND RECIPROCAL (CROSS NO 34.175)

(Formulas: $P\ E\ Rk \times P\ e\ rk$ and $P\ e\ rk \times P\ E\ Rk$)

Parental colors	Parental genotypes	Expected ratios in progenies	Progenies tested	Segregants: Self-colored buff ($P\ E\ Rk$)	Segregants: Self-colored testaceous ($P\ E\ rk$)	Segregants: Buff eye ($P\ e\ Rk$)	Segregants: Testaceous eye ($P\ e\ rk$)	Segregants: Total	Probability values
F₁ generation				F₂ generation					
Buff:									
Cross no. 34.162	$P \frac{E}{e} \frac{Rk}{rk}$	9 3 3 1	14	206	67	75	32	380	0 31
Cross no. 34 175	$P \frac{E}{e} \frac{Rk}{rk}$	9 3 3 1	1	17	4	6	1	28	0 84
Total .	$P \frac{E}{e} \frac{Rk}{rk}$	9 3 3 1	15	223	71	81	33	408	0 39

TABLE 6

WHITE KIDNEY × RED KIDNEY, CROSS NO. 30.015

(Formula: $p\ M\ Rk \times P\ m\ rk$)

Parental colors	Parental genotypes	Expected ratios in progenies	Progenies tested	Segregants: Mottled on buff ($P\ M\ Rk$)	Segregants: Mottled on testaceous ($P\ M\ rk$)	Segregants: Buff ($P\ m\ Rk$)	Segregants: Testaceous ($P\ m\ rk$)	Segregants: White (p)	Segregants: Total	Probability values
F₁ generation				F₂ generation						
Purple/brown	$\frac{P}{p}\ \frac{M}{m}\ \frac{Rk}{rk}$	27.9 9:3.16	2	53	20	18	6	36	133	0.99
F₂ generation				F₃ generation						
Mottled on buff	$\frac{P}{p}\ \frac{M}{m}\ \frac{Rk}{rk}$	27.9.9.3·16	5	83	0	19	9	31	142	Low*
	$\frac{P}{p}\ \frac{M}{m}\ Rk$	9 0.3 0 4	2	27	.	10	..	13	50	0.95
	$\frac{P}{p}\ M\ Rk$	3 0·0.0.1	3	76	..	.		22†	98	0.29
	$P\ M\ Rk$	all	3	100	..	..	..	.	100	...
Mottled on testaceous	$\frac{P}{p}\ \frac{M}{m}\ rk$	0 9 0:3:4	2	19	0	.	14	5	38	Low
Buff	$\frac{P}{p}\ m\ \frac{Rk}{rk}$	0·0:9:3.4	1	..	..	29	7	7‡	43	0.30
	$\frac{P}{p}\ m\ Rk$	0:0.3·0.1	1	..	..	25	..	10	35	0.64
	$P\ m\ \frac{Rk}{rk}$	0·0:3:1:0	2	..	..	37	18	.	55	0.19
Testaceous	$P\ m\ rk$	0:0·0:all	1	.	..	..	73	..	73	...
White	p	0:0:0:0.all	4		..	..	.	123	123	....

in Red Kidney is due to the expression of a recessive gene, the dominant allelomorph being buff, which is represented by the symbol *Rk* (buff) *rk* (testaceous). A recessive gene *e* is responsible for eye pattern demonstrated first by Emerson (*3*). Its dominant allelomorph, *E* makes beans self-colored. There is no indication of linkage between *Rk* and *E*.

WHITE KIDNEY × RED KIDNEY

(Formula: *p M Rk* × *P m rk*)

The F_1 in this cross was mottled purple/buff. The white parent therefore carried *M* and *Rk* and one of the two parents carried *Bl;* it is impossible to know which, because the *Bl* reaction is not evident in either testaceous or white beans. In F_2 there were a number of purple-mottled types ranging from bluish to dark red. These colors were not described accurately enough in the author's original notes to enable one to follow the segregation of *Bl* or its modifiers. The genes segregating in this cross were *P, M,* and *Rk*. The results are shown in table 6. Since all genotypes homozygous for *p* are white, the *M* and *Rk* genes can only be followed in three-fourths of the population. The expected ratio for this cross is 27 *P M Rk* mottled on buff : 9 *P M rk* mottled on testaceous : 9 *P m Rk* buff : 3 *P m rk* testaceous : 16 *p* white. In an F_2 population of 133, a probability value of 0.99 was obtained, when fitted to this ratio. In F_3, five progenies from mottled on buff were segregating for *P, M,* and *Rk*. No mottled-on-testaceous beans were found in a population of 142 although there were 9 self-colored testaceous. The absence of this mottled class made a very poor fit for the expected ratio. Perhaps some mottled-on-testaceous beans were misclassified. Two F_2 mottled-on-buff types segregated for *P* and *M*. Three segregated for *P* and three bred true.

Only two mottled-on-testaceous F_2 plants were submitted to progeny tests. Both segregated for *P* and *M*. The results here are spurious because the mottled offspring were all expected to be mottled on testaceous. There were none of this class but there were 19 mottled on buff which were not expected. This discrepancy may have been due to misclassification of the F_2 plant. The buff F_2 plants could segregate for *P* and *Rk* or breed true. Four were tested in F_3. One segregated for *P* and *Rk;* one for *P;* and one for *Rk*. One testaceous F_2 plant and four whites bred true in F_3.

The results of this cross show segregation for *P, M,* and *Rk*. In F_3 progeny tests, the number of plants mottled on testaceous background (*P M rk*) was usually low. This low number may have been due to misclassification but it is possible that the presence of modifiers altered the segregation of *P M rk* types.

TABLE 7

White Kidney × Red Kidney, Cross No. 32.026

(Formula: *p M Rk bl* × *P m rk Bl*)

Parental colors	Parental genotypes	Expected ratios in progenies	Progenies tested	Segregants								Probability values
				Purple/buff (*P M Rk Bl*)	Purple/testaceous (*P M rk Bl*)	Red/buff (*P M Rk bl*)	Red/testaceous (*P M rk bl*)	Buff (*P m Rk*)	Testaceous (*P m rk*)	White (*p*)	Total	
F_1 generation				F_2 generation								
Purple/buff: Heliotrope slate/pinkish buff.... ..	$\frac{P}{p}\ \frac{M}{m}\ \frac{Rk}{rk}\ \frac{Bl}{bl}$	81:27:27.9.36:12:64	12	218	76	69	19	101	40	165	688	0.71
F_2 generation				F_3 generation								
Testaceous	*P m rk*	0:0·0:0 0 all	1				..		38		38	

WHITE KIDNEY × RED KIDNEY

(Formula: *P M Rk bl* × *P m rk Bl*)

This cross is the same as the one just discussed; the colors in the F_2 were more carefully classified so the segregation of *Bl* could be followed. Table 7 gives a summary of the results. This summary, however, fails to show all the variability encountered. Some phenotype classes contain several colors. The purple/buff class had 54 dark Yvette violet/pinkish buff, 56 Urania blue/pinkish buff, and 108 Ramier blue/pinkish buff, making a total of 218 plants. The purple/testaceous class consisting of 76 plants included 52 analine black/testaceous and 24 dark Corinthian purple/ocher red. The red/buff class had 69 plants which were divided into 19 dark vinaceous-purple/pinkish buff and 50 vinaceous-purple/pinkish buff. The color names indicate that these beans were purple. They showed a slight tinge of purple but were predominantly red. The other colored classes were more uniform, all the red/testaceous were classified as oxblood red/testaceous, all the buff as light pinkish cinnamon, all testaceous as testaceous, and white as white.

There were no F_3 progeny tests made in this cross so it is not possible to say whether the classification made was absolutely correct. Four independent genes were segregated, namely, *P*, *M*, *Rk*, and *Bl*.

GLOYER'S CROSS, WHITE KIDNEY × RED KIDNEY

(Formulas: *p C Rk* × *p c rk* and *P c rk* × *p C Rk*)

In 1928, Gloyer (5) reported on a cross between these two varieties. Inasmuch as the *Rk* gene was encountered, his results are given in table 8. He made no attempt to classify the genotypes, so this has been done from his data. The F_1 was mottled brown/buff; it might be supposed, therefore, that White Kidney contributed *M* and *Rk*. In the F_2, however, the segregation was 103 mottled: 102 self-colored: 56 white. This is much nearer a 6:6:4 ratio than to a 9:3:4. The mottling, then, was due to a heterozygous gene like Lamprecht's *C*. The F_1 was brown/buff; and, since brown/buff, brown/red, buff, red, and white were obtained in F_2, this cross obviously segregated for *Rk* as well as for *P* and *C*. In the F_2 a number of brown segregates were found—bronze, brown, dark brown, and seal brown. In F_3 there was no consistency in the way these brown beans segregated. Bronze, for instance, segregated into bronze, brown, and seal brown, but so did seal brown. For purposes of classification the browns may be grouped together. This classification undoubtedly oversimplifies the situation as will appear later. The browns may be considered to be homozygous for *C*. They may be either *P C Rk* or *P C rk*. The

TABLE 8

ANALYSIS OF GLOYER'S CROSS, WHITE KIDNEY × RED KIDNEY AND RECIPROCAL
(Formulas: $p\,C\,Rk \times P\,c\,rk$ and $P\,c\,rk \times p\,C\,Rk$)

Parental colors	Parental genotypes	Expected ratios in progenies	Progenies tested	Segregants: Brown/buff ($P\frac{C}{c}Rk$)	Segregants: Brown/red ($P\frac{C}{c}rk$)	Segregants: Brown ($P\,C\,Rk$ or $P\,C\,rk$)	Segregants: Buff ($P\,c\,Rk$)	Segregants: Red (testaceous) ($P\,c\,rk$)	Segregants: White (p)	Total	Probability values	Natural hybrids: Number	Natural hybrids: Per cent
	F₁ generation					F₂ generation							
Brown, buff	$\frac{P}{p}\frac{C}{c}\frac{Rk}{rk}$	18 6.12.9.3 16	4	66	37	54	30	18	56	261	0 03		. .
	F₂ generation					F₃ generation							
Brown/buff .	$\frac{P}{p}\frac{C}{c}\frac{Rk}{rk}$	18 6·12 9 3 16	4	29	14	29	9	3	40	124	0 05	2	1.6
	$\frac{P}{p}\frac{C}{c}Rk$	6·0 3 3 0 4	4	25		9	5		10	49	0 19		. .
	$P\frac{C}{c}\frac{Rk}{rk}$	6.2 4 3 1 0	4	44	9	25	17	4		99	0 54	10	1.0
	$P\frac{C}{c}Rk$	2:0 1 1 0:0	6	77		53	24		.	154	0 01	1	0.7
	$P\frac{C}{c}Rk$*	2 0 1.1 0 0	1	22		8	12		.	42	0 66	..	..
Brown/red . . .	$\frac{P}{p}\frac{C}{c}rk$	0:6 3 0 3 4	6		57	33	.	36	46	172	0 68	..	. .
	$P\frac{C}{c}rk$	0 2.1.0 1:0	3		22	12	.	16	.	50	0.50	..	.

Seal brown.	$\frac{P}{p}$ C Rk	0.0 3:0 0.1	2			24	.	..	10	34	0.57	..	..
	. †	. . ..	2		..	46	12	3	..	62	....	1	1.6
	P C Rk	0:0 all	2	..	.	22	.	..	..	22	....	2	9.1
Bronze....	$\frac{P}{p}$ C Rk	0:0.3.0 0 1	5	..	..	92		..	32	124	0.84	1	1.6
	†	. ...	1	..	..	41	3	1	10	55	...	..	...
	P C Rk	0 0.all	1	..	..	54	..	..	..	54	...	2	3.7
Buff	$\frac{P}{p}$ c $\frac{Rk}{rk}$	0 0 0 9.3:4	1	..	..	.	12	11	1	24	Low‡	1	4.2
	P c $\frac{Rk}{rk}$	0:0:0.3.1·0	2	..	.	.	50	17	..	67	0.95	2	3.0
	P c $\frac{Rk§}{rk}$	0:0:0:3 1 0	1	..	..	.	29	7	..	36	0.46	1	2.8
	P c Rk	0 0:0:all	3	..			84	..	..	84	. .	3	3.6
Red (testaceous)	$\frac{P}{p}$ c rk	0.0.0:0.3:1	1	..	.	.	..	23	13	36	0.13	..	...
	P c rk	0:0.0:0:all	2		.			67	..	67	....	.	...
White................	p	0:0:0:0.0.all	12	.	..	.		..	491	491	....	3	0 6

* This plant was recorded as buff in F_2 but bred as brown/buff in F_3.
† This type of breeding behavior is not expected on the hypothesis made to explain the results. See discussion in the text.
‡ The word "low" is used in those cases where probability value is less than 0.01.
§ This plant was recorded as brown/buff in F_2 but bred as buff in F_3.

buff and red colors are homozygous for *c*, buff being *P c Rk* and red, *P c rk*. Furthermore, in the mottled beans the brown/buff is *P Cc Rk* and the brown/red *P Cc rk*. Whites, of course, are homozygous for *p*.

Using these assumptions, the F_2 of this cross should segregate as follows: 18 *P Cc Rk* brown/buff: 6 *P Cc rk* brown/red: 12 *P C Rk* and *P C rk* brown: 9 *P c Rk* buff: 3 *P c rk* red: 16 *p* white. The probability value, when the F_2 data on 261 plants were fitted to this theoretical ratio, was 0.03.

Gloyer presented F_3 and F_4 data. In his tables he included plants which were obviously different in color from the major part of the populations. These few cases should be disregarded because they were undoubtedly due to natural hybridization in the field. In a total population of 1,810 F_3 plants, 28 of these off-types were obtained. This would be 1.5 per cent natural cross-pollination. F_4 data need not be considered here because they were presented in such a way that one cannot judge whether the F_4 progenies were from single plants. Some were numerically so large as to preclude such an assumption. The data on F_3 plants are also summarized in table 8. If the assumptions made to explain the F_2 ratios are correct, the F_3 ratios should fall into certain patterns. The mottled types should not breed true but should segregate mottled and self-colored in the ratio of 1:1.

In the brown/buff, segregation for *P*, *C*, and *Rk* is expected. Four progenies segregated for these three genes. Four progenies also segregated for *P* and *C*. In these cases no red or mottled-on-red beans are expected. Four progenies segregated for *C* and *Rk*. Here no whites are expected. Six progenies segregated for only *C*, giving 1 brown: 2 brown/buff: 1 buff. The fit to this ratio was not very close, the probability value being 0.01. One progeny test of a buff F_2 behaved as a brown/buff segregating for *Cc*. It was probably misclassified in F_2.

F_3 progenies from the brown/red should have segregated for only *P* and *C*. No buff or buff-mottled beans are expected in any of the progenies. Six progenies segregated for *P* and *C* and three for *C* only.

The brown F_2 were grouped into two color classes, seal brown and bronze. If all browns are classed together the breeding behavior is similar in both types. According to the assumptions here proposed by the present author these browns are of the constitution *P C Rk* or *P C rk*. They should segregate for the *P* gene only. As a matter of fact, some buffs and reds appeared in the progeny tests—a result not expected on these assumptions because buffs and reds are both homozygous for *c*. In order to segregate these colors the brown beans would have to be *P Cc Rk rk*; but brown beans cannot be heterozygous for *C*, for these are al-

ways mottled. It is likely, therefore, that these assumptions will have to be amplified to explain the breeding behavior of the brown segregates. Since Gloyer recognized a number of brown colors presumably due to other modifiers this is not fatal to the remainder of the hypothesis.

The buff plants may segregate for *P* and *Rk*. One segregated for both and two for *Rk* only. In addition, one progeny test was made of a brown/buff F_2 which behaved as a buff in F_3, segregating for *Rk*. The red (testaceous) plants should segregate for *P* or breed true. One segregated for *P* and two bred true. The whites, being *p* white, should all breed true and the results of twelve progeny tests agreed with the expectation. However, 3 plants with colored beans were found in 491 white F_3 plants; these were undoubtedly due to natural hybridization.

The assumption of three segregating genes, *P*, *C*, and *Rk* will explain most of the results of this cross. A supplementary hypothesis must be made to explain the breeding behavior of some brown beans. This, however, is beyond the purpose of this review which was made to show that the *Rk* gene has been noted in the literature but the relation between the dominant and recessive allelomorphs was not recognized.

WHITE KIDNEY × NAGAZURA

White segregates taken from F_3 White Kidney × Red Kidney, were used as parents in crosses with Nagazura. The white used as the pistillate parent in cross 34.160 was a segregate in an F_3 population from a buff F_2 (table 6). This buff was segregating for *P* and *Rk*. These whites could be *p m Rk* or *p m rk*. The presence of *Bl* or *bl* could not be told since *Bl* does not modify the color of buff beans. The white used as the pistillate parent in cross 34.161 segregated from a purple/buff F_2 plant (table 6). This white should be *p M Rk Bl* since the F_3 test showed segregation for *P* only. These two whites differed in that one carried *M*, the other *m*. F_2 populations of these crosses showed there were actually four white genotypes: *p m rk Bl*, *p m Rk Bl*, *p M rk Bl*, and *p M Rk Bl*. The results of these genotypes used in crosses with Nagazura (*P M Rk bl*) are shown in tables 9–11.

The third type of segregation was hardly expected because in the F_3 family in which the white parental strain appeared there were no testaceous or testaceous mottled beans, so they were assumed to be *Rk*. Possibly the F_3 population was not large enough to recover the *rk* genotypes. All the whites used were homozygous for *Bl*.

In cross 34.160*a* (formula: *p m rk Bl* × *P M Rk bl*), four genes should segregate. The F_2 data fitted to a 81:27:27:9:36:12:64 ratio gave a probability value of 0.27. The results of F_2 and F_3 are summarized in

TABLE 9

White Kidney × Nagazura, Cross No. 34.160a

(Formula: $p\ m\ rk\ Bl \times P\ M\ Rk\ bl$)

Parental colors	Parental genotypes	Expected ratios in progenies	Progenies tested	Segregants: Purple/buff ($P\ M\ Rk\ Bl$)	Purple/testaceous ($P\ M\ rk\ Bl$)	Red/buff ($P\ M\ Rk\ bl$)	Red/testaceous ($P\ M\ rk\ bl$)	Buff ($P\ m\ Rk$)	Testaceous ($P\ m\ rk$)	White (p)	Total	Probability values
F_1 generation				F_2 generation								
Purple/buff: Mars violet/buff.... ..	$\frac{P}{p}\ \frac{M}{m}\ \frac{Rk}{rk}\ \frac{Bl}{bl}$	81:27:27.9.36:12:64	3	43	16	20	3	20	5	22	129	0.27
F_2 generation				F_3 generation								
Purple/buff: Madder brown/testaceous*.......	$\frac{P}{p}\ \frac{M}{m}\ \frac{Rk}{rk}\ \frac{Bl}{bl}$	81:27:27.9:36:12.64	1	4	0	3	4	2	0	4	17	Low†
Indian purple/light pinkish cinnamon...	$\frac{P}{p}\ M\ \frac{Rk}{rk}\ \frac{Bl}{bl}$	27:9·9·3.0:0:16	1	13	4	3	3		.	7	30	Low
Slate purple/pinkish buff.. . .	$\frac{P}{p}\ M\ \frac{Rk}{rk}\ \frac{Bl}{bl}$	27:9.9.3·0:0 16	3	16	5	3	2		..	4	30	
Slate purple/pinkish buff.....	$\frac{P}{p}\ \frac{M}{m}\ Rk\ \frac{Bl}{bl}$	27:0.9:0.12 0.16	1	16		4		4	..	6	30	Low
Slate purple/pinkish buff	$\frac{P}{p}\ \frac{M}{m}\ \frac{Rk}{rk}\ Bl$	27:9:0:0.9 3 16	1	3	1		.	0	2	4	10	0.05

Taupe brown/light pinkish cinnamon	$\frac{P}{p} M \frac{Rk}{rk} Bl$	9.3·0:0.0 0 4	1	8	7					6	21	0.28
Slate purple/pinkish buff	$\frac{P}{p} M \frac{Rk}{rk} Bl$	9:3:0 0·0 0 4	1	7	2	.		.		5	14	
Indian purple/light pinkish cinnamon	$\frac{P}{p} M\ Rk \frac{Bl}{bl}$	9·0·3·0:0 0 4	1	7		2				4	13	0.88
Slate purple/pinkish buff . . .	$\frac{P}{p} \frac{M}{m} Rk\ Bl$	9:0:0.0 3.0 4	1	4	..	..		6	..	1	11	Low
Slate purple/pinkish buff . .	$P \frac{M}{m} \frac{Rk}{rk} \frac{Bl}{bl}$	27 9 9.3 12.4 0	5	15	1	9	8	9	3	..	45	Low
Slate purple/pinkish buff ..	$P\ M \frac{Rk}{rk} \frac{Bl}{bl}$	9 3 3 1·0 0.0	1	21	7	5	2	..	..	..	35	0.92
Slate purple/pinkish buff . .	$P \frac{M}{m} Rk \frac{Bl}{bl}$	9 0.3.0.4·0 0	1	3	..	3	.	1	..	.	7	0 92
Taupe brown/light pinkish cinnamon	$P \frac{M}{m} Rk \frac{Bl}{bl}$	9·0 3.0·4 0 0	1	8	.	1	..	5	.	..	14	
Taupe brown/light pinkish cinnamon. .	$P \frac{M}{m} \frac{Rk}{rk} Bl$	9:3·0 0 3.1·0	1	5	0		.	4	1	.	10	0.02
Slate purple/pinkish buff.	$P \frac{M}{m} \frac{Rk}{rk} Bl$	9.3 0:0·3 1.0	1	2	1	.		3	2		8	
Taupe brown/light pinkish cinnamon . . .	$P \frac{M}{m} Rk\ Bl$	3:0.0.0·1 0 0	1	3				3		.	6	0.17

* The colors marked with an asterisk are not in conformity with the breeding behavior in the progeny tests; they may be due to error in the color classification in the F_2.

† The word "low" is used in those cases where the probability value is less than 0 01.

TABLE 9—(*Continued*)
White Kidney × Nagazura, Cross No. 34.160*a*
(Formula: $p\ m\ rk\ Bl \times P\ M\ Rk\ bl$)

Parental colors	Parental genotypes	Expected ratios in progenies	Progenies tested	Segregants: Purple/buff ($P\ M\ Rk\ Bl$)	Purple/testaceous ($P\ M\ rk\ Bl$)	Red/buff ($P\ M\ Rk\ bl$)	Red/testaceous ($P\ M\ rk\ bl$)	Buff ($P\ m\ Rk$)	Testaceous ($P\ m\ rk$)	White (p)	Total	Probability values
	F₂ generation						F₃ generation					
Purple/testaceous:												
Madder brown/testaceous	$\frac{P}{p}\ \frac{M}{m}\ rk\ \frac{Bl}{bl}$	0 27.0:9·0.12 16	1		2		1	.	3	3	9	0.50
Indian purple/light pinkish cinnamon*.	$\frac{P}{p}\ \frac{M}{m}\ rk\ \frac{Bl}{bl}$	0:27:0:9.0:12.16	1	.	4	.	3	..	3	3	13	
Indian purple/light pinkish cinnamon*	$\frac{P}{p}\ M\ rk\ \frac{Bl}{bl}$	0:9:0:3:0:0:4	1	..	2		6	..	..	2	10	Low
Madder brown/testaceous	$P\ \frac{M}{m}\ rk\ \frac{Bl}{bl}$	0:9:0:3.0.4:0	1	..	2	..	3	..	3	.	8	0.19
Indian purple/light pinkish cinnamon*.	$P\ M\ rk\ \frac{Bl}{bl}$	0·3·0:1.0 0 0	1		4	..	2	..	..	..	6	0.65
Red/buff:												
Deep hellebore red/light pinkish cinnamon	$\frac{P}{p}\ \frac{M}{m}\ Rk\ bl$	0:0:9:0 3:0.4	2		..	15	..	5	..	7	27	0.83
Light red/light pinkish cinnamon .	$\frac{P}{p}\ \frac{M}{m}\ Rk\ bl$	0.0:9:0.3 0 4	2		..	24	..	9	..	8	41	
Oxblood red/light pinkish cinnamon	$\frac{P}{p}\ M\ \frac{Rk}{rk}\ bl$	0 0 9·3·0·0·4	1		.	4	3	..	..	4	11	0 43
Deep hellebore red/light pinkish cinnamon	$\frac{P}{p}\ M\ Rk\ bl$	0:0.3·0:0 0·1	1			4		..	.	1	5	0.80

Oxblood red/jasper red*	$P \frac{M}{m} \frac{Rk}{rk} bl$	0·0·9:3:3.1:0	1			4	1	2	0		7	0 93
Oxblood red/light pinkish cinnamon .	$P \frac{M}{m} \frac{Rk}{rk} bl$	0:0:9:3:3 1.0	2	.	.	15	6	3	1		25	
Oxblood red/light pinkish cinnamon. .	$P\ M \frac{Rk}{rk} bl$	0.0.3.1.0:0.0	1	.	.	10	1			.	11	0.40
Slate purple*/light pinkish cinnamon....	$P \frac{M}{m} Rk\ bl$	0:0.3·0:1·0.0	1	..	..	7	.	3			10	0.90
Deep hellebore red/light pinkish cinnamon	$P \frac{M}{m} Rk\ bl$	0 0 3·0:1·0 0	2	..	.	9		2		..	11	
Oxblood red/light pinkish cinnamon .	$P\ M\ Rk\ bl$	0 0·all	1	..	.	10		.	.		10	....
Red/testaceous:												
Madder brown/testaceous .. .	$\frac{P}{p} \frac{M}{m} rk\ bl$	0·0:0 9·0:3:4	3	..	..	.	22	.	14	7	43	0.03
Deep hellebore red/light pinkish cinnamon*...	$\frac{P}{p} \frac{M}{m} rk\ bl$	0:0:0:9:0.3 4	1	..	.		4	..	3	2	9	
Oxblood red/jasper red	$\frac{P}{p} M\ rk\ bl$	0.0 0·3·0 0·1	4				25			10	35	0 93
Madder brown/testaceous	$\frac{P}{p} M\ rk\ bl$	0 0:0:3.0 0 1	1				17	.		3	20	
Madder brown/testaceous. ..	$P \frac{M}{m} rk\ bl$	0.0:0:3:0:1.0	1				9		3		12	0.99
Madder brown/testaceous . .	$P\ M\ rk\ bl$	0:0:0.all	1				8				8	.

* The colors marked with an asterisk are not in conformity with the breeding behavior in the progeny tests, they may be due to error in the color classification in the F_2.
† The word "low" is used in those cases where the probability value is less than 0 01

table 9. There were a number of colors in F_3 which were grouped in the following way: purple/buff included raisin black/pinkish buff (plate 1, fig. 9); Indian purple/pinkish buff (plate 1, fig. 10); and dark heliotrope slate/pinkish buff (plate 1, fig. 11). Purple/testaceous included raisin black/testaceous (plate 1, fig. 12) and Indian purple/testaceous (plate 1, fig. 13). Red/buff included oxblood red/pinkish buff (plate 1, fig. 15), maroon/pinkish buff (plate 1, fig. 14); and deep hellebore red/pinkish buff (plate 1, fig. 16). Red/testaceous included oxblood red/testaceous (plate 1, fig. 22) and maroon/testaceous (plate 1, fig. 17). Buff was classed as pinkish cinnamon (plate 1, fig. 7), testaceous as testaceous (plate 1, fig. 1) and white as white (plate 1, fig. 33).

The F_2 color descriptions in general were similar to those of F_3. In one case madder brown was chosen as the standard color. This was an unfortunate choice because both reddish purple and reds were classed in this group. Had colors been chosen a little farther off this borderline description, the F_3 results would appear more convincing. As it is, some madder-brown mottled beans showed in their breeding behavior to be carrying *Bl* and others were homozygous for *bl*. Progeny tests also showed that *P M Rk* and *P M rk* genotypes were not always clearly distinguishable. Some beans described as mottled on buff bred as mottled on testaceous and vice versa. F_3 progeny tests of the purple/buff phenotype showed that one F_2 plant was segregating for *P*, *M*, *Rk*, and *Bl*. The poor fit is due to the small number of 17 plants, where 256 were needed to recover all genotypes. Four segregated for *P*, *Rk*, and *Bl*. Here a poor fit was obtained because fewer red/buff types appeared than were expected. One segregated for *P*, *M*, and *Bl;* one for *P*, *M*, and *Rk;* two for *P* and *Rk;* one for *P* and *Bl;* one for *P* and *M;* five for *M*, *Rk*, and *Bl;* one for *Rk* and *Bl;* two for *M* and *Bl;* two for *M* and *Rk;* and one for *M*.

Of the purple/testaceous F_2 plants subjected to progeny tests, two segregated for *P*, *M*, and *Bl;* one for *P* and *Bl;* and one for *Bl*. In the F_3 progenies from red/buff four segregated for *P* and *M;* one for *P* and *Rk;* one for *P*, three for *M* and *Rk;* one for *Rk;* three for *M;* and one bred true. Four progenies of red/testaceous segregated for *P* and *M;* five for *P;* one for *M;* and one bred true.

With some exceptions, probably caused by misjudgment of color of F_2 plants, these results bear out the assumption that the white parent was *p m rk Bl*. In some cases the size of the population in F_3 was too small for very good agreement with expectancy.

In the F_1 of cross 34.160*b* (formula: *p m Rk Bl* $\times$ *P M Rk bl*), *Rk* was homozygous, so no mottled-on-testaceous or self-colored testaceous beans were expected. Three genes were segregating in F_2, *P*, *M*, and *Bl*,

giving a 27 :9 :12 :16 ratio. The F_2 results fitted to this ratio gave a probability value of 0.13.

The purple/buff F_2 plants were slate-purple/pinkish buff and Indian purple/light pinkish cinnamon (plate 1, fig. 10). Both gave results in conformity with expectation. This phenotype may segregate for *P, M,* and *Bl.* Two segregated for *P, M,* and *Bl;* three for *P* and *M;* three for *P* and *Bl;* two for *M* and *Bl;* four for *P;* two for *Bl;* two for *M;* and one bred true. The red/buff phenotypes were oxblood red/light pinkish cinnamon (plate 1, fig. 15) and light red/light pinkish cinnamon. This phenotype should segregate for only *P* and *M.* Three progenies segregated for *P* and *M;* four for *M;* and five bred true. These results, as shown in table 10, are all in conformity with the assumption that the white parent was *p m Rk Bl.*

In cross 34.161*a* (formula: *P M rk Bl* $\times$ *P M Rk bl*), *M* was homozygous so no self-colored types were expected. The segregating genes were *P, Rk,* and *Bl.* The F_2 results from two progenies of this cross were as follows: Purple/buff, 73; purple/testaceous, 12; red/buff, 26; red/testaceous, 8; and white, 26. Fitted to the theoretical ratio of 27 :9 :9 :3 : 16 there should be in a population of 145, 61.2 purple/buff, 20.4 purple/testaceous, 20.4 red/buff, 6.8 red/testaceous, and 36.2 white plants. The probability value for such a fit is 0.07. No F_3 progeny tests were made of this cross.

In cross 34.161*b* (formula: *p M Rk Bl* $\times$ *P M Rk bl*), *M* and *Rk* were both homozygous; therefore segregation for only *P* and *Bl* was expected in the hybrid. No self-colored or mottled beans with a testaceous background were expected. The results of this cross are presented in table 11. The F_2 results fitted to a 9 :3 :4 ratio gave a probability value of 0.01, owing to the small number of white beans. However, the results from F_3 fitted the expectations very well. The purple/buff were all slate purple/pinkish buff. Four progenies in F_3 segregated for *P* and *Bl;* two for *P;* four for *Bl;* and one bred true. The red/buff which were classified as oxblood red/light pinkish cinnamon in F_2 should have segregated for *P* or bred true because *M* and *Rk* were homozygous. Two progenies tested in F_3 segregated for *P* and five bred true.

The experiments with the whites of known genotypes bear out the conclusions made in the earlier tests. Mottling is due to the presence of a single gene, *M;* in the presence of its recessive allelomorph *m,* the beans are self-colored. The *Rk* gene can be distinguished in both mottled and self-colored beans. In mottled beans the ground color is buff if *Rk* is present and testaceous if *rk* is present; in self-colored beans *Rk* is buff and *rk* is testaceous. *Bl* may be carried in white, testaceous, and buff

TABLE 10
White Kidney × Nagazura, Cross No. 34.160b
(Formula: $p\ m\ Rk\ Bl \times P\ M\ Rk\ bl$)

Parental colors	Parental genotypes	Expected ratios in progenies	Progenies tested	Segregants: Purple/buff ($P\ M\ Rk\ Bl$)	Red/buff ($P\ M\ Rk\ bl$)	Buff ($P\ m\ Rk$)	White (p)	Total	Probability values
F₁ generation				**F₂ generation**					
Purple/buff: Mars violet/buff	$\frac{P}{p}\ \frac{M}{m}\ Rk\ \frac{Bl}{bl}$	27.9.12.16	2	33	17	21	15	86	0.13
F₂ generation				**F₃ generation**					
Purple/buff: Slate purple/pinkish buff	$\frac{P}{p}\ \frac{M}{m}\ Rk\ \frac{Bl}{bl}$	27:9:12:16	2	18	7	3	11	39	0 36
Slate purple/pinkish buff	$\frac{P}{p}\ \frac{M}{m}\ Rk\ Bl$	9:0:3 4	2	12	.	3	6	21	0.28
Slate purple/pinkish buff	$\frac{P}{p}\ \frac{M}{m}\ Rk\ Bl$	9 0 3 4	1	6		1	4	11	
Slate purple/pinkish buff	$\frac{P}{p}\ M\ Rk\ \frac{Bl}{bl}$	9:3:0 4	2	11	5		6	22	0.90
Indian purple/light pinkish cinnamon	$\frac{P}{p}\ M\ Rk\ \frac{Bl}{bl}$	9.3:0.4	1	17	3		5	25	
Slate purple/pinkish buff	$\frac{P}{p}\ M\ Rk\ Bl$	3·0·0.1	1	12	..	.	2	14	0.83
Slate purple/light pinkish cinnamon	$\frac{P}{p}\ M\ Rk\ Bl$	3:0:0:1	2	19		.	8	27	
Indian purple/light pinkish cinnamon	$\frac{P}{p}\ M\ Rk\ Bl$	3:0:0:1	1	9	.	..	3	12	

Slate purple/pinkish buff	$P\,\frac{M}{m}\,Rk\,\frac{Bl}{bl}$	9:3:4:0	2	20	3	10	..	33	0.36
Indian purple/light pinkish cinnamon	$P\,M\,Rk\,\frac{Bl}{bl}$	3.1:0:0	2	17	4	..	..	21	0 54
Slate purple/pinkish buff	$P\,\frac{M}{m}\,Rk\,Bl$	3:0:1:0	2	24	..	4	..	28	0.19
Slate purple/pinkish buff	$P\,M\,Rk\,Bl$	All	1	13	..	.	..	13	....
Red/buff:									
Oxblood red/light pinkish cinnamon	$\frac{P}{p}\,\frac{M}{m}\,Rk\,bl$	0:9.3.4	2	.	17	8	6	31	0.79
Light red/light pinkish cinnamon	$\frac{P}{p}\,\frac{M}{m}\,Rk\,bl$	0 9:3:4	1	.	11	2	4	17	
Oxblood red/light pinkish cinnamon	$\frac{P}{p}\,M\,Rk\,bl$	0 3 0 1	2		21	.	4	25	0 30
Oxblood red/light pinkish cinnamon	$P\,\frac{M}{m}\,Rk\,bl$	0 3.1 0	4		25	9		34	0.85
Oxblood red/light pinkish cinnamon	$P\,M\,Rk\,bl$	0 all	5		32			32	...

TABLE 11
WHITE KIDNEY × NAGAZURA, CROSS NO. 34.161b
(Formula: $p\ M\ Rk\ Bl \times P\ M\ Rk\ bl$)

Parental colors	Parental genotypes	Expected ratios in progenies	Progenies tested	Segregants: Purple/buff ($P\ M\ Rk\ Bl$)	Segregants: Red/buff ($P\ M\ Rk\ bl$)	Segregants: White (p)	Segregants: Total	Probability values
F1 generation				**F2 generation**				
Purple/buff: Mars violet/buff	$\frac{P}{p}\ M\ Rk\ \frac{Bl}{bl}$	9 3 4	4	174	75	60	309	0.01
F2 generation				**F3 generation**				
Purple/buff: Slate purple/pinkish buff	$\frac{P}{p}\ M\ Rk\ \frac{Bl}{bl}$	9.3.4	4	37	13	13	63	0.26
Slate purple/pinkish buff	$\frac{P}{p}\ M\ Rk\ Bl$	3·0:1	2	13	..	5	18	0.79
Slate purple/pinkish buff	$P\ M\ Rk\ \frac{Bl}{bl}$	3 1 0	4	38	11	..	49	0.69
Slate purple/pinkish buff	$P\ M\ Rk\ Bl$	All	1	12	.	.	12	...
Red/buff: Oxblood red/light pinkish cinnamon	$\frac{P}{p}\ M\ Rk\ bl$	0 3 1	2	..	22	4	26	0 26
Oxblood red/light pinkish cinnamon	$P\ M\ Rk\ bl$	0.all	5	.	60	.	60	..

beans with no modification in the expression of color. When *Bl* is added to a red-mottled bean the mottling is changed from red to purple. There is no indication of linkage between *M, Bl,* or *Rk.*

RED KIDNEY × CHINA RED, AND RECIPROCAL

(Formulas: *P r rk Bl* × *P R Rk bl* and *P R Rk bl* × *P r rk Bl*)

Since both of these varieties were colored they were homozygous for *P*. The China Red is a dark-red bean (plate 1, fig. 6) which matches very closely Ridgway's (*18*) oxblood red or is even a little darker—Victoria lake. The F_1 plants of these crosses were all mottled. If this mottling were due to a heterozygous color gene the ratio of mottled to self-colored in F_2 should be 1:1; actually it was 134:107. In the F_2 segregants eight color types were obtained. Among these were the familiar buff and testaceous. This cross then, was segregating for *Rk*. The presence of *Rk* explains the two ground colors, buff and testaceous in the mottled beans. The action of *Bl* can also be seen in the mottled beans, some being purple mottled, others red. Two other self-colors were obtained in F_2: purple (plate 1, figs. 23, 24) and oxblood red (plate 1, fig. 25). To account for these, the assumption was made that China Red has a red gene similar to, if not identical with Lamprecht's (*14*) *R* which produces mottling when heterozygous; Red Kidney is homozygous for *r*. Segregation for three genes explained the results if the following assumptions for the genotypes were made: *Rr Rk Bl* is purple/buff; *Rr rk Bl* is purple/testaceous; *Rr Rk bl* is red/buff and *Rr rk bl* is red/testaceous. Purple may be *R Rk Bl* or *R rk Bl;* oxblood red may be *R Rk bl* or *R rk bl;* buff may be *r Rk Bl* or *r Rk bl;* and testaceous, *r rk Bl* or *r rk bl.* The expected ratio should be 18:6:6:2:12:4:12:4, respectively, for the eight colors. These assumptions were tested in progenies from F_2 plants. All mottled F_2 beans should segregate mottled and self-colored in the ratio of 1:1. The colors would depend on the interaction of *Rk* and *Bl.*

The purple/buff F_2 should segregate for *R, Rk,* and *Bl.* Since the mottling is due to *Rr,* no mottled types should breed true. It is possible, however, for some to be homozygous for *Rk* or *Bl.* Some F_2 progenies bred in F_3 as purple/buff but the colors noted for the F_2 plants were not purple/buff. These plants are indicated in table 12 by an asterisk following the color which is not in conformity with the breeding behavior.

Seventeen F_2 purple/buff segregated for *R, Rk,* and *Bl* in F_3; six segregated for *R* and *Rk;* three for *R* and *Bl;* and four for *R.*

Thirteen F_2 purple/testaceous plants were tested in F_3; since *rk* was present in this genotype segregation for only *R* and *Bl* was expected. Nine segregated for *R* and *Bl* and four for *R.*

TABLE 12

Red Kidney × China Red (Crosses Nos. 34.007 and 33.051) and Reciprocal (Cross No. 34.008)

(Formulas: $P\ r\ rk\ Bl \times P\ R\ Rk\ bl$ and $P\ R\ Rk\ bl \times P\ r\ rk\ Bl$)*

Parental colors	Parental genotypes	Expected ratios in progenies	Progenies tested	Segregants: Purple/buff ($\frac{R}{r}$ Rk Bl)	Purple/testaceous ($\frac{R}{r}$ rk Bl)	Red/buff ($\frac{R}{r}$ Rk bl)	Red/testaceous ($\frac{R}{r}$ rk bl)	Purple (R Bl)	Oxblood red (R bl)	Buff (r Rk)	Testaceous (r rk)	Total	Probability values
F₁ generation				F₂ generation									
Purple/buff:													
Hay's maroon/cinnamon, cross no. 34.007	$\frac{R}{r}\frac{Rk}{rk}\frac{Bl}{bl}$	18:6:6:2:12:4:12:4	3	26	14	16	8	20	5	21	6	116	
Hay's maroon/cinnamon, cross no. 34.008	$\frac{R}{r}\frac{Rk}{rk}\frac{Bl}{bl}$	18:6:6:2:12:4:12:4	4	28	15	7	4	15	8	16	4	100	0.22
Hay's maroon/cinnamon, cross no. 33.051	$\frac{R}{r}\frac{Rk}{rk}\frac{Bl}{bl}$	18.6:6.2:12:4.12:4	1	9	2	5	0	1	3	3	2	25	
F₂ generation				F₃ generation									
Purple/buff:													
Hay's maroon/cinnamon	$\frac{R}{r}\frac{Bk}{rk}\frac{Bl}{bl}$	18:6:6:2:12:4:12:4	9	39	15	15	4	28	8	25	7	141	
Dull purplish black/vinaceous cinnamon	$\frac{R}{r}\frac{Rk}{rk}\frac{Bl}{bl}$	18:6:6:2.12:4:12:4	1	9	3	1	0	1	1	3	2	20	
Dark Yvette violet/pinkish cinnamon	$\frac{R}{r}\frac{Rk}{rk}\frac{Bl}{bl}$	18:6:6:2:12.4.12:4	1	0	0	2	2	2	3	1	0	10	0.56
Dull purplish black/ferruginous†	$\frac{R}{r}\frac{Rk}{rk}\frac{Bl}{bl}$	18:6:6:2:12 4:12:4	3	4	3	4	0	6	0	7	3	27	
Oxblood red†/pinkish cinnamon	$\frac{R}{r}\frac{Rk}{rk}\frac{Bl}{bl}$	18:6:6:2.12:4:12:4	2	6	5	7	0	6	5	8	0	37	
Hessian brown/ferruginous†	$\frac{R}{r}\frac{Rk}{rk}\frac{Bl}{bl}$	18:6:6:2:12:4.12:4	1	2	1	2	0	3	1	1	1	11	

Hay's maroon/cinnamon	$\frac{R}{r}\ \frac{Rk}{rk}\ Bl$	6:2:0:0:4:0·3:1	2	12	4		.	7	.	8	0	31	
Dull purplish black/ferruginous†..	$\frac{R}{r}\ \frac{Rk}{rk}\ Bl$	6:2:0:0:4·0.3:1	2	12	2	..	.	11	..	8	5	38	0.11
Hessian brown/ferruginous†	$\frac{R}{r}\ \frac{Rk}{rk}\ Bl$	6.2·0:0·4:0:3·1	1	13	0	..	..	4	..	0	5	22	
Raisin black/warm buff .	$\frac{R}{r}\ \frac{Rk}{rk}\ Bl$	6:2:0·0·4:0 3:1	1	2	0	..	..	3	..	0	1	6	
Hessian brown/ferruginous†	$\frac{R}{r}\ Rk\ \frac{Bl}{bl}$	6.0:2.0 3 1.4·0	1	5	..	4	..	4	1	4	..	18	
Oxblood red†/pinkish cinnamon	$\frac{R}{r}\ Rk\ \frac{Bl}{bl}$	6·0.2:0.3 1.4:0	1	6	..	2	..	4	1	0	..	13	0.49
Raisin black/warm buff	$\frac{R}{r}\ Rk\ \frac{Bl}{bl}$	6.0:2:0:3:1:4:0	1	0	..	..	..	3	1	4	..	8	
Hay's maroon/cinnamon	$\frac{R}{r}\ Rk\ Bl$	2:0:0.0 1.0:1.0	2	10	..	..	..	5	..	9	.	24	
Dull purplish black/vinaceous cinnamon	$\frac{R}{r}\ Rk\ Bl$	2 0·0:0 1.0:1.0	1	3	..	.	..	0	..	3	..	6	0.18
Dull violet black/pinkish cinnamon	$\frac{R}{r}\ Rk\ Bl$	2·0·0:0 1.0 1:0	1	5		..		1	..	2		8	
Purple/testaceous· Dull purplish black/ferruginous.	$\frac{R}{r}\ rk\ \frac{Bl}{bl}$	0.6 0·2.3 1.0 4	4	..	22	.	6	13	4		13	58	
Victoria lake/testaceous. ...	$\frac{R}{r}\ rk\ \frac{Bl}{bl}$	0:6:0:2:3.1·0.4	3	.	19		5	11	3		9	47	0.81
Hessian brown/ferruginous	$\frac{R}{r}\ rk\ \frac{Bl}{bl}$	0·6.0:2:3.1:0.4	2	.	16		10	10	3		12	51	

* All genotypes in the table are homozygous for *P*.

† The colors marked with a dagger are not in conformity with the breeding behavior in the progeny tests; they may be due to error in the color classification in the F_2.

TABLE 12—(*Continued*)
Red Kidney × China Red (Crosses Nos. 34.007 and 33.051) and Reciprocal (Cross No. 34.008)
(Formulas: $P\ r\ rk\ Bl \times P\ R\ Rk\ bl$ and $P\ R\ Rk\ bl \times P\ r\ rk\ Bl$)*

Parental colors	Parental genotypes	Expected ratios in progenies	Progenies tested	Segregants: Purple/buff $(\frac{R}{r}\ Rk\ Bl)$	Purple/testaceous $(\frac{R}{r}\ rk\ Bl)$	Red/buff $(\frac{R}{r}\ Rk\ bl)$	Red/testaceous $(\frac{R}{r}\ rk\ bl)$	Purple $(R\ Bl)$	Oxblood red $(R\ bl)$	Buff $(r\ Rk)$	Testaceous $(r\ rk)$	Total	Probability values
F₂ generation				F₃ generation									
Purple/testaceous: —*Continued*													
Hessian brown/ferruginous... ...	$\frac{R}{r}\ rk\ Bl$	0.2 0:0.1·0.0·1	3	.	20			21			9	50	
Dull purplish black/ferruginous..	$\frac{R}{r}\ rk\ Bl$	0 2 0·0 1:0.0.1	1	..	5	.		1	..		2	8	0.08
Red/buff:													
Oxblood red/pinkish cinnamon.......	$\frac{R}{r}\ \frac{Rk}{rk}\ bl$	0 0 6 2.0.4:3·1	3	..	..	21	3	..	4	13	0	41	
Oxblood red/testaceous†	$\frac{R}{r}\ \frac{Rk}{rk}\ bl$	0.0 6·2 0 4:3:1	4	..	..	17	11	..	10	6	7	51	0.71
Amaranth purple/pinkish cinnamon.	$\frac{R}{r}\ \frac{Rk}{rk}\ bl$	0 0 6.2:0.4.3:1	1	..	.	7	1	..	4	3	1	16	
Bordeaux/testaceous†	$\frac{R}{r}\ \frac{Rk}{rk}\ bl$	0.0 6 2 0 4 3:1	2	.		6	4	.	8	5	0	23	
Amaranth purple/pinkish cinnamon.	$\frac{R}{r}\ Rk\ bl$	0 0 2·0 0.1 1·0	1	..	..	15	..	..	10	5	..	33	
Bordeaux/testaceous†............	$\frac{R}{r}\ Rk\ bl$	0:0.2.0·0 1.1.0	4	.	.	41	.	.	27	27	.	95	0.34
Oxblood red/pinkish cinnamon. ...	$\frac{R}{r}\ Rk\ bl$	0·0:2:0 0.1:1:0	2		.	17	.	.	11	5	..	33	

Red/testaceous:													
Oxblood red/testaceous..............	$\frac{R}{r}$ *rk bl*	0:0:0:2:0:1:0:1	6		..	.	41		25	..	24	90	0.87
Bordeaux/testaceous	$\frac{R}{r}$ *rk bl*	0.0:0·2:0:1:0:1	1		.		3	..	0	.	0	3	
Purple:													
Dull purplish black	*R* $\frac{Bl}{bl}$	0.0.0.0:3:1:0:0	10		.		.	129	46	.	..	175	0.70
Raisin black	*R* $\frac{Bl}{bl}$	0:0 0:0 3:1.0:0	1	.	.	..	..	15	1	..	..	16	
Liver brown... . .	*R* $\frac{Bl}{bl}$	0:0 0.0.3:1.0:0	2		..	.	.	25	6	..	..	31	
Dull purplish black	*R Bl*	0:0:0:0:all	4		..	..	..	82	..	..	..	82	....
Liver brown.......	*R Bl*	0·0·0·0:all	1	.	..	..	.	11	..	..	..	11	....
Oxblood red:													
Oxblood red.......	*R bl*	0:0:0:0:0:all	4		..	..	.	..	61	.	..	61	....
Pompeian red......	*R bl*	0:0·0.0 0·all	8	.	..	.	..	..	151	.	..	151	....
Bordeaux	*R bl*	0:0.0:0:0·all	1	.	..	..	..	..	4	.	..	4	....
Buff:													
Vinaceous cinnamon	*r* $\frac{Rk}{rk}$	0:0:0:0.0:0 3·1	3	.	..	..	.	..	.	38	15	53	0.99
Light pinkish cinnamon....... . ..	*r* $\frac{Rk}{rk}$	0 0 0.0.0 0 3.1	2			.			.	22	5	27	
Vinaceous cinnamon	*r Rk*	0:0·0.0·0.0.all	2						.	32		32	....
Testaceous....	*r rk*	0:0:0:0.0:0:0:all	2			..	..	.	..	..	18	18	....

* All genotypes in the table are homozygous for *P*.

† The colors marked with a dagger are not in conformity with the breeding behavior in the progeny tests; they may be due to error in the color classification in the F_2.

Seventeen F_2 red/buff were tested in F_3. Some of these had been misclassified as red/testaceous in F_2. Since red/buff is *Rr Rk bl* segregation for only *R* and *Rk* is possible. Ten progenies segregated for *R* and *Rk*, and seven for *R*.

Seven red/testaceous F_2 plants were tested in F_3. This genotype, being *Rr rk bl*, should segregate for *R* only, giving 1 *R rk bl* oxblood red: 2 *Rr rk bl* red/testaceous: 1 *r rk bl* testaceous. The numbers obtained were 25:44:24. If the assumptions made were correct, the purple F_2 should segregate for *Bl* only. Thirteen progenies segregated for *Bl* and five bred true. All oxblood red phenotypes should breed true. The thirteen tested conformed to expectation. The buff phenotypes should segregate for *Rk* or breed true. Five segregated for *Rk* and two bred true. Only two testaceous F_2 were grown in F_3 and they bred true as expected.

CHINA RED × RED KIDNEY

(Formula: *P R rk bl* × *P r rk Bl*)

In this cross the F_1 was purple/testaceous rather than purple/buff as in the crosses reported in table 12. The China Red in this cross must have been *P R rk bl* while in the others it was *P R Rk bl*. The F_2 was in conformity with expectations in that no buff or mottled-on-buff beans appeared. The F_3 progenies grown from F_2 plants were all homozygous for *rk*. Seventeen purple/testaceous F_2 plants were tested in F_3. Eight of them segregated for *R* and *Bl* and nine segregated for *R* only. Seven red/testaceous segregated for *R* as expected. Five purple F_2 segregated in F_3 for *Bl* and three bred true. One oxblood red bred true as did three testaceous.

The color of China Red, then, is due to the presence of *R*. This variety may carry *Rk* (table 12) or *rk* (table 13). In all crosses there was segregation for the purple gene *Bl*, the dominant allelomorph coming from Red Kidney. Only one strain of China Red has been used in the crosses but the results found here prove that within the variety there are at least two genotypes. Since the presence of *Rk* does not alter the color of the bean, this genetic variation in the variety cannot be detected by examination of the beans themselves.

CHINA RED × GENEVA RED KIDNEY

The F_1 and F_2 data from this cross are given in table 14. The Geneva Red Kidney is a testaceous segregate from the cross White Kidney × Red Kidney made by Gloyer (5). A summary of his results regarding color segregation was given in table 8. Owing to its origin, this variety may carry brown modifiers, at least *c*, and probably others which could

TABLE 13
CHINA RED × RED KIDNEY, CROSS NO. 34.173
(Formula: $P\ R\ rk\ bl \times P\ r\ rk\ Bl$)*

Parental colors	Parental genotypes	Expected ratios in progenies	Progenies tested	Segregants: Purple/testaceous ($\frac{R}{r}\ rk\ Bl$)	Segregants: Red/testaceous ($\frac{R}{r}\ rk\ bl$)	Segregants: Purple ($R\ Bl$)	Segregants: Oxblood red ($R\ bl$)	Segregants: Testaceous ($r\ rk$)	Total	Probability values
F_1 generation				**F_2 generation**						
Purple/testaceous. Victoria lake/testaceous	$\frac{R}{r}\ rk\ \frac{Bl}{bl}$	6:2:3·1:4	2	35	14	10	4	28	91	0.23
F_2 generation				**F_3 generation**						
Purple/testaceous: Victoria lake/testaceous....	$\frac{R}{r}\ rk\ \frac{Bl}{bl}$	6.2 3:1:4	8	23	11	17	4	9	64	0.12
Victoria lake/testaceous...	$\frac{R}{r}\ rk\ Bl$	6.0:1:0:1	9	53		31	..	20	104	0.31
Red/testaceous: Oxblood red/testaceous .	$\frac{R}{r}\ rk\ bl$	0·2.0:1.1	7		51		32	22	105	0 38
Purple: Victoria lake	$R\ rk\ \frac{Bl}{bl}$	0·0:3:1:0	5			56	21	..	77	0 65
Victoria lake	$R\ rk\ Bl$	0.0:all	3			49	..		49	. .
Oxblood red	$R\ rk\ bl$	0·0:0:all	1	.			4		4	.
Testaceous .	$r\ rk$	0.0:0.0:all	3					34	34	

* All genotypes in the table are homozygous for P.

TABLE 14

China Red × Geneva Red Kidney, Cross No. 34.177

Parental colors	Progenies tested	Segregants											
		Black	Liver brown	Corinthian purple	Chestnut brown	Mahogany red	Hay's russet	Deep Corinthian red	Carmine	Oxblood red	Vinaceous fawn	Testaceous	Total
F₁ generation		F₂ generation											
Liver brown	7		33	4	7	3	11	3	..	3	6	12	82
F₂ generation		F₃ generation											
Corinthian purple	1	3		1								..	4
	1					4			3	5		1	13
Hay's russet	1	1					1						2
	1							2	13	2		3	20
	1						18	5	9	1		12	45
	2						12		3	1		2	18
	1						17				..	12	29
	1						11			..	..		11
Deep Corinthian red	1							6			..	2	8
Oxblood red	1						2		7	2		1	12
Vinaceous fawn	1					1				1		18	20
	5										..	27	27
Testaceous	4											53	53

very likely give different results from those obtained with other Red Kidney beans.

The F_1 of this cross was not mottled so that neither the *R* nor the *C* genes were segregating. This is rather disconcerting because the presence of *R* has been shown to be the reason for the oxblood red color in the China Red variety in crosses with other strains of Red Kidney. This cross was different in other respects as well. In the F_2 population of 82 plants, nine colors were recognized: Corinthian purple (plate 2, fig. 46); liver brown (plate 2, fig. 47); chestnut-brown (plate 2, fig. 48); mahogany red; Hay's russet (plate 2, fig. 49) deep Corinthian red (plate 2, fig. 50); oxblood red (plate 2, fig. 51); vinaceous-fawn (plate 2, fig. 52); and testaceous (plate 2, fig. 53). The occurrence of liver brown and Corinthian purple indicates that *Bl* is segregating; similarly the brown segregates indicates the presence of some brown color modifiers. Oxblood red was reclaimed only three times in the population, hinting that its expression is due to a double recessive condition of two genes; the number expected for a 15:1 ratio in a population of 82 is 5.1. Only one oxblood red was tested in F_3. This one did not breed true as expected on the above hypothesis. It segregated 1 Hay's russet and 1 testaceous in a population of 12.

The vinaceous-fawn color was proved to be a variation of testaceous because F_3 progenies from this color all bred true for testaceous except one which had 1 oxblood red and 1 mahogany red plant in a population of 20. This could be explained as a natural outcross in the F_2 generation. The vinaceous-fawn and testaceous colors, then, can be combined in F_2, giving a total of 18 plants. This is about one-fourth of the population. The number expected on a 3:1 ratio is 20.5; this fits the expected ratio with a probability value of 0.53.

Hay's russet showed in the F_3 tests that it may segregate both oxblood red and testaceous colors. It, therefore, carries brown modifiers which are able to alter the colors in red beans.

It is interesting to note that seven of the ten F_3 progeny rows tested, segregated testaceous. The number of testaceous plants segregated in these seven progenies was 33 in a total population of 145. When these data are fitted to a 3:1 ratio the probability value is 0.54.

DARK RED KIDNEY × RED KIDNEY

(Formula: *P R Rk Bl* × *P r rk bl*)

This cross segregated like the crosses involving China Red. Dark Red Kidney is reddish purple classed as Indian purple (plate 1, fig. 4). It should therefore carry *Bl*. Since this cross segregated purple and red

TABLE 15

DARK RED KIDNEY × RED KIDNEY, CROSS NO. 34.167

(Formula: $P\ R\ Rk\ Bl \times P\ r\ rk\ bl$)*

Parental colors	Parental genotypes	Expected ratios in progenies	Progenies tested	Segregants: Purple/buff ($\frac{R}{r}$ Rk Bl)	Purple/testaceous ($\frac{R}{r}$ rk Bl)	Red/buff ($\frac{R}{r}$ Rk bl)	Red/testaceous ($\frac{R}{r}$ rk bl)	Indian purple (R Bl)	Oxblood red (R bl)	Buff (r Rk)	Testaceous (r rk)	Total	Probability values
F1 generation				F2 generation									
Purple/buff. Raisin black/avellaneous	$\frac{R}{r}\frac{Rk}{rk}\frac{Bl}{bl}$	18:6:6:2:12:4:12.4	12	113	41	60	0	111	24	86	23	458	Low†
F2 generation				F3 generation									
Purple/buff: Dark perilla purple/avellaneous	$\frac{R}{r}\frac{Rk}{rk}\frac{Bl}{bl}$	18:6.6:2.12:4.12:4	10	29	6	9	4	28	4	18	13	111	0.10
Dark perilla purple/avellaneous	$\frac{R}{r}$ Rk $\frac{Bl}{bl}$	6:0:2:0·3:1:4:0	3	9		5		6	1	2		23	0.45
Dark perilla purple/avellaneous	$\frac{R}{r}\frac{Rk}{rk}$ Bl	6:2:0:0·4:0:3·1	2	2	4			2	..	2	2	12	0.10
Dark perilla purple/avellaneous	$\frac{R}{r}$ Rk Bl	2·0 0:0·1:0:1:0	4	12	..	.	..	8	.	4		24	0.50
Purple/testaceous: Raisin black/Corinthian red	$\frac{R}{r}$ rk $\frac{Bl}{bl}$	0:6:0:2:3·1:0·4	7	..	28	..	9	13	8	..	12	70	0.30
Raisin black/Corinthian red	$\frac{R}{r}$ rk Bl	0.2 0:0.1:0:0.1	8	..	42	..	..	26	..	..	26	94	0.60

Red/buff:													
Bordeaux/light pinkish cinnamon.	$\frac{R}{r}\frac{Rk}{rk}bl$	0:0:6:2·0:4:3:1	1	.	..	3	0	..	3	3	1	10	0.73
Oxblood red/light pinkish cinnamon	$\frac{R}{r}\frac{Rk}{rk}bl$	0:0 6:2 0:4:3:1	2	..	..	7	2	.	4	4	2	19	
Bordeaux/light pinkish cinnamon	$\frac{R}{r}Rk\ bl$	0:0:2:0:0·1:1:0	4	..	..	15	.	..	9	5	.	29	0 98
Oxblood red/light pinkish cinnamon	$\frac{R}{r}Rk\ bl$	0:0:2·0:0.1:1.0	5	..	..	22	..		10	12	.	44	
Red/testaceous:													
Bordeaux/light pinkish cinnamon	$\frac{R}{r}rk\ bl$	0.0:0.2·0.1:0·1	1	..			4	.	2		2	8	0.99
Indian purple:													
Raisin black .	$R\frac{Bl}{bl}$	0:0 0:0.3.1.0:0	5	..				46	11	.		57	0 40
Indian purple .	$R\frac{Bl}{bl}$	0.0 0 0 3:1 0 0	7					55	17			72	
Indian purple ..	$R\ Bl$	0:0:0.0·all	3					29		.		29	...
Raisin black	$R\ Bl$	0 0·0.0.all	10				.	91				91	..
Oxblood red:													
Victoria lake .	$R\ bl$	0·0.0 0.0 all	10						74			74	

* All genotypes in the table are homozygous for P.

† The word "low" is used in those cases where the probability value is less than 0.01.

self-colored beans it must be assumed that *bl* came from Red Kidney. The segregation of *Bl* is somewhat surprising because in all other crosses involving Red Kidney *Bl* is present. The results of this cross are summarized in table 15. In F_2 purple/buff was described as raisin black/avellaneous and in F_3 as raisin black/light pinkish cinnamon (plate 1, fig. 26). The purple/testaceous class was described as raisin black/Corinthian red in F_2 and as Indian purple/testaceous in F_3 (plate 1, fig. 13). The red/buff phenotype was divided into Bordeaux/light pinkish cinnamon and oxblood red/light pinkish cinnamon; in F_3 they were all described as oxblood red/light pinkish cinnamon (plate 1, fig. 21). No red/testaceous beans were recognized in the F_2; in F_3, they were labeled oxblood red/testaceous (plate 1, fig. 27). The purple phenotype included raisin black and Indian purple in F_2, dull violet-black (plate 1, fig. 23), and Indian purple (plate 1, fig. 29). The red beans were described as Victoria lake in F_2 and as oxblood red (plate 1, fig. 25) in F_3. Buff was called light pinkish cinnamon in F_2 and pinkish cinnamon (plate 1, fig. 7) in F_3. The testaceous (plate 1, fig. 1) phenotype was called by that color name in both F_2 and F_3 generations.

The F_2 population of 458 plants gave a poor fit for the expected ratio. This was because of the fact that one phenotype, red/testaceous, was not recognized. This was clearly an error in classification because one of the thirteen red/buff F_2 plants subjected to F_3 progeny test proved to be a red/testaceous genotype. In the purple/buff F_2 plants grown in F_3 ten segregated for *R*, *Rk*, and *Bl*; three for *R* and *Bl*; two for *R* and *Rk*; and four for *R* only. Fifteen purple/testaceous F_2 plants were tested. Seven segregated for *R* and *Bl*, and eight for *R* only. Twelve red/buff plants were grown in F_3. Three segregated for *R* and *Rk* and nine for *R* only. The single red/testaceous plant tested in F_3 was classed as a red/buff in F_2; it segregated for *R*.

In the purple phenotypes two shades of purple were distinguished in F_2, raisin black and Indian purple. In breeding behavior these were identical. Five raisin black F_3 progenies segregated for *Bl* and ten bred true; seven Indian purple progenies segregated for *Bl* and three bred true. The oxblood red group was called Victoria lake in F_2; however, in F_3 ten progenies bred true for oxblood red. No buff or testaceous F_2 plants were grown in F_3.

LONG ROMAN × CHINA RED, AND RECIPROCAL

(Formulas: *P M R Rk bl* × *P m R Rk bl* and *P m R Rk bl* × *P M R Rk bl*)

In the cross, Red Kidney × Long Roman (table 3) the red/buff Long Roman (plate 1, fig. 3) was found to be of the genetic constitution *P M*

TABLE 16

LONG ROMAN × CHINA RED (CROSS NO. 33.063) AND RECIPROCAL (CROSS NO. 33.070)
(Formulas: *P M R Rk bl* × *P m R Rk bl* and *P m R Rk bl* × *P M R Rk bl*)*

Parental colors	Parental genotypes	Expected ratios in progenies	Progenies tested	Segregants: Red/buff ($M\ R\ Rk\ bl$)	Segregants: Oxblood red ($m\ R\ Rk\ bl$)	Segregants: Total	Probability values
F₁ generation				F₂ generation			
Red/buff Bordeaux/pale pinkish buff cross no. 33.063	$\frac{M}{m}\ R\ Rk\ bl$	3:1	3	132	54	186	0.20
Bordeaux/pale pinkish buff cross no. 33.070	$\frac{M}{m}\ R\ Rk\ bl$	3:1	3	100	34	134	0.90
Total	$\frac{M}{m}\ R\ Rk\ bl$	3:1	6	232	88	320	0.30
F₂ generation				F₃ generation			
Red/buff Bordeaux/pale pinkish cinnamon	$\frac{M}{m}\ R\ Rk\ bl$	3:1	1	7	1	8	0.43
Amaranth purple/pale pinkish cinnamon	$M\ R\ Rk\ bl$	all:0	1	17		17	
Oxblood red Bordeaux	$m\ R\ Rk\ bl$	0:all	2		68	68	
Pompeian red	$m\ R\ Rk\ bl$	0:all	1		18	18	

* All genotypes in the table are homozygous for *P*

Rk bl. The China Red has been found to be *P R Rk bl* (table 12) and *P R rk bl* (table 13). Since genes for two types of mottling, *Rr* and *M*, have been shown in these two varieties it was hoped that an interaction of the two could be seen in this cross. In that case the F_1 should show double mottling and the F_2 should segregate into 6 double mottled *Rr M* : 2 mottled *Rr* : 6 mottled *M* : 2 self-colored *r m* and *R m*.

However, in this reciprocal cross shown in table 16, the F_1 was red/buff and in F_2 there was segregation for mottled and self-colored oxblood red in the ratio of 3:1, with a probability value of 0.30, for a population of 320. On the basis of a 3:1 ratio there should be 80 reds. There were actually 88 so that a 14:2 ratio is very improbable.

As expected, there is no segregation for *Bl* since both varieties have been shown to be homozygous for *bl*.

In the F_2, four colors were recognized: the red/buff phenotype was classified as Bordeaux/pale pinkish cinnamon and amaranth purple/pale pinkish cinnamon; and the red phenotype was divided into Bordeaux and Pompeian red. In the F_3 progeny tests there was overlapping of the colors so that in table 16 they are grouped in only two classes, red/buff and red.

Only four F_2 plants were grown in F_3. One red/buff segregated for *M* and one bred true. Both oxblood reds bred true.

Since no testaceous phenotypes arose in this cross both varieties must have been homozygous for *Rk;* and since no purple plants were found they were both homozygous for *bl*. The genotype of the China Red may then be written, *P m R Rk bl;* and for Long Roman, *P M R Rk bl*. The presence of *R* in Long Roman, however, was not detected in crosses with Red Kidney (table 3). The poor fit to expectation in the crosses reported there, however, was due to misfits of two mottled classes red/buff and red/testaceous. Segregation for *M* was 256 mottled : 78 self-colored; expected, 250.5 :83.5. Had *R* been segregating simultaneously, the ratio of mottled to self-colored should have been 292.25 :41.75. It seems probable that the red mottling is due to the interaction of *M* and *R*. To explain all the facts presented here *M* and *R* would have to be linked. Such a linkage of *M* and *R* offers a workable hypothesis as to the nature of the red in red-mottled beans. In the previous crosses the cause of the red mottling was not discussed; *M* was considered to be a gene which restricts the expression of the darker color in bicolored beans, and it has been shown to be independent of *Rk* and *Bl*. If *M* and *R* were linked, the following color types would be expected: purple/buff would be *P MR Rk Bl* or $P\ \frac{mR}{mr}\ Rk\ Bl$; purple/testaceous, *P MR rk Bl* or $P\ \frac{mR}{mr}\ rk\ Bl$;

red/buff, $P\ MR\ Rk\ bl$ or $P\ \frac{mR}{mr}\ Rk\ bl$; red/testaceous, $P\ MR\ rk\ bl$ or $P\ \frac{mR}{mr}\ rk\ bl$; purple, $P\ mR\ Rk\ Bl$ or $P\ mR\ rk\ Bl$; oxblood red, $P\ mR\ Rk\ bl$ or $P\ mR\ rk\ bl$. Thus the oxblood red color in both self-colored and red-mottled beans could be due to the same gene, *R*. If there is a linkage between *M* and *R* it must be very strong because no cross-overs have been noted. The crosses reported in tables 1, 2, and 3 are between red/buff mottled beans and Red Kidney. Assuming linkage, these crosses may be represented as $P\ MR\ Rk\ bl \times P\ mr\ rk\ Bl$; the F_1 would be $\frac{P}{P}\ \frac{MR}{mr}\ \frac{Rk}{rk}\ \frac{bl}{Bl}$. The cross-over classes would be *mR* and *Mr;* of these, the *mR* cross-overs should be easily identified as self-colored oxblood red, $P\ mR\ bl$, or self-colored purple, $P\ mR\ Bl$. Since none of these appeared in any of these crosses, the crossing-over would have to be very small, if any. This argument is faulty for the same reason that Emerson's (*3*) *YZ* mottling theory was. Tjebbes (*24*) found close linkage between a red gene *R* and a striping gene *S* which occurs in the Cranberry variety. His red gene is not the same as the one encountered here because when heterozygous it does not produce mottling. It is probable, also, that *M* and *S* are not identical. It seems to be a strange coincidence that two red genes should each be linked with a mottling gene. More critical data are needed to study the interaction of *M* and *R*.

CHINA RED × MEXICAN RED, AND RECIPROCAL

Both these varieties are dark red, matching Ridgway's (*18*) oxblood red very closely. Mexican Red, however, has a black hilum ring. The F_1 was slightly darker than either parent and was classed as Victoria lake although it was more purple than this color. The hilum ring was black. In F_2, 52 mottled beans were found in a population of 364. These mottled ones were not recognized until some mottled beans appeared in F_3. The remnant F_2 seed was then reëxamined and the F_2 results given in table 17 are based on the second examination. It is possible therefore that some F_2 seed which were grown in F_3, although described as self-colored, were actually mottled. Unfortunately no remnant seed was available for those F_2 plants submitted to progeny tests. As is indicated in table 17 some mottled beans appeared in F_3 from all three colors tested. From Victoria lake, two progenies out of eighteen tested segregated 5 mottled and 26 self-colored; from oxblood red three progenies out of twenty-five segregated 11 mottled and 37 self-colored; and from Vandyke red five progenies out of twenty-seven tested segregated 25

TABLE 17

SEGREGATION FOR SEED-COAT COLOR IN CHINA RED × MEXICAN RED (CROSS NO. 34.172) AND RECIPROCAL (CROSS NO. 34.176)

Parental colors	Parental color of hilum ring	Progenies tested	Segregants: Victoria lake/testaceous	Oxblood red/testaceous	Pompeian red/testaceous	Victoria lake	Perilla purple	Oxblood red	Pompeian red	Ocher red	Total
F₁ generation			F₂ generation								
Victoria lake:											
Cross no. 34.172	Black	10		4	28	29	3	93	82	15	254
Cross no. 34.176	Black	6	1	14	5	15	8	39	21	7	110
Total	Black	16	1	18	33	44	11	132	103	22	364
F₂ generation			F₃ generation								
Victoria lake	Black	1	4			12		2		.	18
		1		.		7	1	5		..	13
		1				2		4	3	.	9
		8				63		35		..	98
		4				36				..	36
Victoria lake	No hilum ring	1	.	1	.	2	.	5	5	..	13
		1			.	4		4	..		8
		1	.		.	5	..	..	..	1	6
Oxblood red	Black	2	.	10	..		..	26	1	..	37
		1		.	.	4	2	9	2	.	17
		2				8		14	3	..	25
		1	.			..		13	1	..	14
		7			.	.	.	74		..	74

Oxblood red	No hilum ring	1	..	1		2	1	5	2	.	11
		2	.			3		17	3	3	26
		3						23	8	6	37
		5						31	7		38
		1	.					11		.	11
Vandyke red	Black	1		8		2		5	4		19
		1				2		5	3		10
		1		3				2	3		8
		2		10				26	8		44
		2		1	3			10	10	3	27
		1						4	4		8
		1						9			9
		1								11	11
Vandyke red	Orange	5						33	18		51
		2							7	22	29
		2								10	10
Vandyke red	No hilum ring	4						13	22	7	42
		2						19	7		26
		1						12		1	13
		1							3	1	4

mottled and 73 self-colored. It is thus apparent the segregation of mottled and self-colored beans in this hybrid is not constant. If the mottling were due to the action of the *R* gene, the self-colored beans should breed true unless some modifying factors prevented the expression of mottling. The mottled types should segregate mottled and self-colored plants in the ratio of 1:1.

The only available data on this question are the results from progeny tests of two F_3 mottled plants. These segregated 10 mottled and 11 self-colored. This information though meager bears out the assumption that the reactions of the *R* gene were obscured in F_1 and partially in F_2 by modifying genes and it was not until these were eliminated in F_3 that clear-cut segregation for *R* could be detected.

The segregation of color of the seed coat in this cross is almost as baffling. The shades of red obtained in the F_2 and F_3 were so numerous and gradual that separation into modal classes was difficult. It is certain, however, that no testaceous beans were obtained in any of the F_2 or F_3 populations. Since China Red has been shown to carry *Rk* (table 12) and *rk* (table 13), and since no testaceous *rk* types appeared in this cross, both parents must have been homozygous for the *Rk* gene. It must be assumed, however, that the presence of other modifying genes prevented the buff *Rk* types from appearing because no buff beans were found in any of the offspring from this cross.

The presence of a colored hilum ring is a difficult character to study because not all beans from a single plant show the hilum ring color. It is therefore very easy to make errors in classification. Furthermore, the color of the hilum ring depends largely on the color of the seed coat. This is demonstrated in the F_2 data. The beans were classed as Victoria lake, perilla purple, oxblood red, Pompeian red, and ocher red. There were 44 plants with Victoria lake seed-coat color, 36 with black hilum ring, 1 with orange ring, and 7 with no ring. All 11 of the plants with perilla purple seed coats had black hilum ring. The oxblood red plants numbered 132 of which 89 had black ring, 15 had orange, and 28 had no ring. The 132 Pompeian red plants were classed as 72 with black hilum ring, 18 with orange, and 13 with no ring. In 22 ocher-red plants, 11 had black hilum ring, and 11 had orange. Segregation for hilum-ring character in this cross is shown in table 18. Some progenies bred true for both black and orange hilum ring but no true-breeding progenies with no hilum ring were obtained. Some of the F_2 plants classed as having no hilum ring proved in progeny tests to have rings. The character for orange hilum ring bred true in two progenies of oxblood red and two of Vandyke red. Black ring seems to be dominant over orange but there

are cases of F_2 plants with orange ring segregating plants with black rings. The genetic nature of the hilum ring character therefore is difficult to understand.

TABLE 18

SEGREGATION FOR HILUM-RING COLOR IN CHINA RED × MEXICAN RED (CROSS NO. 34.172) AND RECIPROCAL (CROSS NO. 34.176)

Parental color	Parental color of hilum ring	Progenies tested	Hilum-ring color of segregants: Black	Orange	No hilum ring	Total segregants
F_1 generation			F_2 generation			
Victoria lake	Black	16	269	47	48	364
F_2 generation			F_3 generation			
Victoria lake	Black	7	59		31	90
		1	5	4		9
		7	75			75
Victoria lake	No ring	2	4	11	4	19
		1	3		5	8
Oxblood red	Black	4	39	15	10	64
		3	31		3	34
		1		10	7	17
		5	52			52
Oxblood red	No ring	6	9	28	37	74
		4		23	6	29
		2		20		20
Vandyke red	Black	5	60	13	17	90
		3	15	6		21
		2	14		3	17
		1	8			8
Vandyke red	Orange	5	6	42	3	51
		4		39		39
Vandyke red	No ring	6	21	42	5	68
		2		17		17

MEXICAN RED × RED KIDNEY

The results from this cross are summarized in table 19. The F_1 was violet carmine. The names given to the color classes varied somewhat between the F_2 and F_3 generations. The relation between them was as follows: Beans which were classified as dull purplish black in F_2 were black and Indian purple in F_3; Victoria lake was used in both generations; in F_2

TABLE 19

MEXICAN RED × RED KIDNEY, CROSS NO. 33.496

Parental color	Progenies tested	Segregants							
		Black	Indian purple	Victoria lake	Chocolate	Oxblood red	Vandyke red	Testaceous	Total
F_1 generation		F_2 generation							
Violet carmine	20		14	254	14	62	34	118	496
F_2 generation		F_3 generation							
Dull purplish black	1	5	4			1		1	11
	1	1	1	2		2			6
	1	3	7		3	1			14
	1	1	3			1			5
	1	1	2	3					6
	1	2	1						3
	1	3							3
	1		1	1	1				3
	2		4		4				8
	2		3						3
Liver brown	1		1	3	1			1	6
	1		2					1	3
	1		2		1	1			4
	2		7			2			9
	1		1	1	1				3
	1		1						1
	1							1	1
	2			2		7	3		12

Oxblood red	2					6	4	2	12
	2					3	5		8
	3					15			15
Mars brown	1				1	1	1	1	4
	1				3			3	6
	1				3		1	1	5
	1				1				1
	1					2			2
	1							3	3
Orange cinnamon.	10							53	53
Ferruginous	6							28	28

liver brown and burnt umber were grouped together as chocolate in F_3; oxblood red was used in both generations; beans classed as Pompeian red in F_2 were called Vandyke red in F_3. A number of light-red types similar to testaceous were distinguished in F_2 but all proved to be testaceous when submitted to progeny tests. These F_2 color names, then, can all be grouped together: orange-cinnamon, Japan rose, ferruginous, and testaceous. F_3 progeny tests were made of a number of F_2 plants but owing to poor stand in the nursery, the F_3 populations were too small to obtain accurate ratios.

Although the colors do not segregate in definable ratios a number of illuminating facts are observed. The colors obtained in this cross are illustrated in plate 2, figures 54–59. The F_1 was purplish red (violet carmine) therefore the *Bl* gene was able to modify the color in this hybrid. It appears that each parent contributed some purple modifying genes because in F_3 progenies from F_2 Indian-purple plants (plate 2, figs. 63–64), some beans appeared which could be described by no better word than black (plate 2, fig. 54). These may well be due to accumulation of darkening modifiers contributed from both parents. The light-red beans, such as Vandyke red (plate 2, fig. 58), may well be due to homozygous combinations of red genes with *bl* and other recessive purple modifiers.

Another interesting fact noted in this cross is the absence of segregation of color of the hilum ring, presumably due to the fact that both Red Kidney and Mexican Red have colored hilum rings; the ring in the former is orange and in the latter, black. On this basis, crosses between Red Kidney and China Red or Dark Red Kidney should have segregated for colored hilum ring. This, however, was not observed; but no special attention was paid to this character in those crosses.

Still another fact is apparent in the Mexican Red × Red Kidney cross. In the F_2 population of 496 plants, there were 118 testaceous plants (table 19). Fitted to a 3:1 ratio there should have been 124; the probability value for such a fit is 0.54. In the F_3 progenies there were 7 which segregated 10 testaceous plants in a population of 47. The probability value for these results, fitting a 3:1 ratio, is 0.57. Thus it is apparent that the recessive gene *rk* is segregating normally in this cross. But what has become of *Rk* since no buff beans were found in any of the progenies? It is now apparent that Mexican Red either has a third allelomorph of the *Rk rk* gene pair or other color genes prevent its expression as buff.

Still another fact is apparent in this cross which should not be overlooked and that is the absence of mottled beans. Since some mottled beans were obtained in the China Red × Mexican Red cross the assump-

tion was made that the *R* gene was segregating. China Red is known to carry the dominant allelomorph *R;* so, if mottling were due to heterozygous *R,* Mexican Red must carry *r*. This fits in with the facts obtained in this cross. Red Kidney has been shown to carry *r* and if Mexican Red does also, no mottled beans are expected in the progeny of this cross.

MEXICAN RED × DARK RED KIDNEY

The F_1 of this cross was classed as Vandyke red with a black hilum ring. In F_2 it segregated for the hilum-ring character. The results of the segregation for seed-coat color are given in table 20 and for the hilum ring character in table 21.

Regarding mottling, much the same result was obtained here as in the China Red × Mexican Red cross. The faint mottling was entirely overlooked in F_2. When the F_3 was obtained showing mottled beans, the F_2 remnants were reëxamined and four mottled beans were found which had been previously classified as Corinthian purple. Two F_2 progenies segregated such a high proportion of mottled offspring in F_3 that they must have been mottled in F_2. These two progenies, which are indicated in table 20, consisted of 42 plants of which 22 were mottled. For a 1:1 ratio there should have been 21. Fitted to such a ratio the probability value is 0.76. It is apparent that the *R* gene is segregating in this cross but some modifying genes prevent the expression of mottling in some cases. Further proof for the presence of *R* was obtained by subjecting known mottled F_3 beans of this cross to progeny tests. From eight progenies 120 plants were harvested, 56 being mottled and 64 self-colored. These data fitted to a 1:1 ratio give a probability value of 0.47.

Since some reddish purple (Indian purple, plate 2, figs. 63, 64), and purple (perilla purple, plate 2, fig. 65) beans were obtained, it is assumed that the color genes in Dark Red Kidney (Indian purple, plate 1, fig. 4) were responsible. No blacks, however, were found in the F_3 so there was no accumulation of dark modifiers as in Mexican Red × Red Kidney. In the cross Dark Red Kidney × Red Kidney (table 15) evidence of segregation of *Bl* was obtained. Thus the *Bl* gene carried by Red Kidney is not the same gene as the purple modifier in Dark Red Kidney or black beans would have been obtained in the F_3 of the cross Mexican Red × Dark Red Kidney. Oxblood red was reclaimed often in the F_2 of this cross and so was a still lighter red, classed as acajou red (plate 2, figs. 69, 70). Acajou red is lighter in color than the Mexican Red parent and indicates that it may have fewer dominant color genes for red or purple than either parental variety. No testaceous beans were obtained in this cross indicating that neither parent carries *rk*.

TABLE 20

SEGREGATION FOR SEED-COAT COLOR IN MEXICAN RED × DARK RED KIDNEY, CROSS NO. 34.171

Parental colors	Parental color of hilum rings	Progenies tested	Segregants								
			Indian purple, Pompeian red	Perilla purple/light perilla purple	Oxblood red/Pompeian red	Acajou red/testaceous	Indian purple	Perilla purple	Oxblood red	Acajou red	Total
F1 generation			F2 generation								
Vandyke red	Black	3		4			14	70	42		130
F2 generation			F3 generation								
Indian purple	Black	1*			3		3		2	2	10
		1	3		4		44		18	1	70
		3			4		45		26		75
		2					24		18		42
Corinthian purple	Black	1*	1	18			2	10	1		32
		1	1				9	8	5	1	24
		6		26	3	1	28	50	20	11	139
		5		9			11	59	11		90
		1		1				24			25
		4					17	21	7		45
		1						25			25
Deep hellebore red	Black	2		12			4	49	17	3	85
		1		1				20			21
		1					1	12	3	1	17
		2						32			32

Oxblood red	Black	1			1				33	1	35
		2							31	15	46
Deep hellebore red	Orange	2		3				15	6	.	24
		1		7				15			22
		1						6	15	8	29
		1						10		7	17
Oxblood red	Orange	2						2	40	20	62
		7							61	27	88
		1							3		3
Indian purple	No ring	2							9	7	16
Corinthian purple	No ring	1					7	29	3		39
Oxblood red	No ring	1					1		4	2	7
		2†					12		4		16
		1						3	9		12
		1							8	5	13
		4							43		43

* An F_2 plant, probably mottled.
† An F_2 plant, probably Indian purple.

In the study of the hilum-ring character, summarized in table 21, the same difficulties of clearly distinguishing the different phenotypes were encountered as described in the China Red × Mexican Red. Here there was further evidence that the color of the hilum ring and the color of the seed coat are associated. This condition has already been noted in the literature (*12, 17*). In the F_2 generation the Indian-purple progeny

TABLE 21

Segregation for Hilum-Ring Color in Mexican Red × Dark Red Kidney, Cross No. 34.171

Parental color	Parental color of hilum ring	Progenies tested	Hilum-ring color of segregants			Total segregants
			Black	Orange	No hilum ring	
F_1 generation			F_2 generation			
Vandyke red	Black	3	82	20	28	130
F_2 generation			F_3 generation			
Indian purple	Black	7	93	39	65	197
Corinthian purple	Black	9	122	42	33	197
		8	114	31		145
		1	11		2	13
		1	25			25
Deep hellebore red	Black	2	54	26	5	85
		3	35	14		49
		1	21			21
Oxblood red	Black	3	53	16	12	81
Deep hellebore red	Orange	2		47	4	51
		3		41		41
Oxblood red	Orange	2	7	45	8	60
		1	1	2		3
		3		37	12	49
		4		41		41
Indian purple	No hilum ring	1			1	1
		1		1	14	15
Corinthian purple	No hilum ring	1	9	16	14	39
Oxblood red	No hilum ring	1	2	2	8	12
		4		17	21	38
		2		14		14
		2			27	27

were classed as 10 with black hilum ring and 4 with no ring; in the perilla purple, 59 had black hilum ring, 9 had orange, and 1 no ring; in the oxblood red, 9 had black hilum ring, 11 had orange, and 22 no ring; in the perilla purple/light perilla purple all 4 had black hilum rings.

The plants with black hilum rings seem to be able to segregate both orange rings and no rings as well as breed true. Orange hilum ring usually breeds true or segregates no ring but occasionally some progeny with black ring come from F_2 plants with orange hilum ring. F_2 plants classed as having no rings did not always breed true—in fact most of them reverted to orange rings or in a few cases black. Two F_3 progenies from oxblood with no hilum ring bred true in F_3.

As can be seen in table 20 there is some discrepancy in the color description of F_2 and F_3. The two colors, Corinthian purple and deep hellebore red recognized in F_2 were grouped together in F_3 as perilla purple.

NATURE OF THE COLOR COMPLEX IN MEXICAN RED

In the three crosses just discussed no attempt was made to give the genetic formula of Mexican Red. We can, however, make some assumptions which are based on the results of the crosses involving this variety.

Both China Red and Dark Red Kidney carry *R* and since some mottled beans were found in F_2 and F_3, when these varieties were used in crosses with Mexican Red, the mottling was attributed to the action of *R*. F_4 data from both China Red × Mexican Red and Mexican Red × Dark Red Kidney crosses were presented to show that mottled hybrid beans do segregate in the ratio of 1 mottled to 1 self-colored as expected. This was explained on the basis that the reactions of *R* were obscured by interactions of other color genes but when these modifiers were eliminated in the F_3 the action of *R* could be readily seen. Thus Mexican Red carries *r*. Since Red Kidney also carries *r* there should be no mottling in the progeny of a cross between these varieties. Since none were found this is further evidence that Mexican Red must carry *r*.

Some F_3 beans were obtained in these crosses which were darker than either parent, a fact explained by assuming that the Mexican Red carries purple modifiers as well as the other varieties and the dark colors are due to accumulation of these modifiers in homozygous condition in some genotypes. The reverse situation was also observed. Some F_3 beans were lighter red than either parent indicating the elimination of the dominant purple modifiers. The genetic nature of the colored hilum ring has not been settled in this work. Because it is difficult to always distin-

guish this character, errors are easily made in classification. After they have been made it is hard to reconcile the notes on the hybrids in the succeeding generation. There is some evidence that the color of the ring is associated with the color of the seed coat but there are also enough exceptions to make a general rule untenable.

Mexican Red was shown in crosses with Red Kidney to carry a dominant allelomorph of *rk* since one-fourth of the F_2 and segregating F_3 progenies were testaceous. China Red and Dark Red Kidney have been shown to carry *Rk* a dominant allel of *rk* which makes beans buff in color in the absence of the dominant red gene *R*. Now, since Mexican Red does not carry *R*, and since no buff beans were found in any of the crosses, it must carry other red color genes which prevent *Rk* from appearing as buff. Another explanation, advanced earlier in the paper is that this variety may carry a third allel in the *Rk rk* series. No critical data are available to make a choice between these two possibilities.

Crosses involving Mexican Red were the most difficult to analyze genetically but the hybrids show more promise as a foundation for a breeding program to improve the color of Red Kidney than any other variety tested. The new light-red colors which may be useful are: Pompeian red (plate 2, fig. 44), and ocher red (plate 2, fig. 45) from the cross China Red × Mexican Red; Vandyke red (plate 2, fig. 58) from the cross Mexican Red × Red Kidney; and acajou red (plate 2, figs. 69, 70) from the cross Mexican Red × Dark Red Kidney. Whether these reds will prove useful to this end remains to be seen in later breeding work.

SUMMARY

Hybrids between red-seeded varieties of common beans were made to study the genetic nature of red. Ridgway's (*18*) color nomenclature was used in the descriptions. The results of this study are applicable in improvement of the Red Kidney variety, which changes in time from red to brown or tan while in storage or when in sunlight. Six genes were encountered which affect seed-coat color or its distribution.

P is a primary pigmentation factor necessary for any color to develop. Beans with *P* but without any complementary pigmentation color genes are white. All the bean varieties studied carried one or more complementary color factors. Beans homozygous for *p* are white regardless of the color genes they may have.

M is a mottling gene which was found in a number of red-mottled varieties; its recessive allelomorph, *m*, is self-colored.

Rk, a gene for buff color, is the dominant allelomorph of *rk*, which is responsible for the testaceous color typical of the Red Kidney variety.

The interactions of *Rk rk* have not been known heretofore. *M Rk* beans are mottled on buff background, and *M rk* are mottled on testaceous background.

R is a gene for deep red (oxblood red). In the genotypes studied *R Rk* and *R rk* were oxblood red; *r Rk*, buff; and *r rk*, testaceous. Beans heterozygous for *R* are mottled: those with *Rr Rk* are mottled on buff background and those with *Rr rk* are mottled on testaceous.

Bl is a color modifier which changes oxblood red to purple. It also changes red-mottled beans to purple-mottled when the mottling is caused by either heterozygous *R* or *M*.

E is the dominant allelomorph of *e*, a gene for eye pattern. *E* beans are self-colored. The eyed variety used was white with a red eye, the red being due to *rk*.

No linkage was found between *Rk M Bl*, *Rk R Bl*, or *Rk E*. Linkage between these genes and *P* could not be demonstrated because all *p* genotypes are white. Some data were obtained indicating complete linkage of *M* and *R*. No cross-over classes were found.

Not one of these genes was suitable as a color modifier of Red Kidney. However, F_3 segregates were obtained in crosses involving Mexican Red which were nearer the ideal type. Further experiments are necessary to ascertain the practical value of these reds. The genotype of Mexican Red was not obtained because it carries a number of modifiers which made classification difficult. No buff segregates were found in crosses involving Mexican Red, although about one-fourth of the F_2 population was testaceous in the cross Mexican Red × Red Kidney. Mexican Red either has modifiers which prevent the expression of buff or it carries a third allelomorph for the *Rk rk* gene pair.

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PLATES

PLATE 1

Parental Varieties:

Fig. 1. Red Kidney 4370.
Fig. 2. Nagazura 4390.
Fig. 3. Long Roman 4521.
Fig. 4. Dark Red Kidney (65)31.
Fig. 5. Speckled Kidney 50(51)30.
Fig. 6. China Red 4414.
Fig. 7. Buff *P Rk*.
Fig. 8. Mexican Red 4437.

F_3 Segregants of White Kidney × Nagazura:

Fig. 9. Raisin black/pinkish buff (*P M Rk Bl*).
Fig. 10. Indian purple/pinkish buff (*P M Rk Bl*).
Fig. 11. Dark heliotrope slate/pinkish buff (*P M Rk Bl*).
Fig. 12. Raisin black/testaceous (*P M rk Bl*).
Fig. 13. Indian purple/testaceous (*P M rk Bl*).
Fig. 14. Maroon/pinkish buff (*P M Rk bl*).
Fig. 15. Oxblood red/pinkish buff (*P M Rk bl*).
Fig. 16. Deep hellebore red/pinkish buff (*P M Rk bl*).
Fig. 17. Maroon/testaceous (*P M rk bl*).
Fig. 18. Oxblood red/orange cinnamon (*P M rk bl*).

F_3 Segregants of China Red × Red Kidney:

Fig. 19. Raisin black/pinkish buff ($P \frac{R}{r} Rk\ Bl$).
Fig. 20. Raisin black/testaceous ($P \frac{R}{r} rk\ Bl$).
Fig. 21. Oxblood red/light pinkish cinnamon ($P \frac{R}{r} Rk\ bl$).
Fig. 22. Oxblood red/testaceous ($P \frac{R}{r} rk\ bl$).
Fig. 23. Dull violet black (*P R Bl*).
Fig. 24. Dull purplish black (*P R Bl*).
Fig. 25. Oxblood red (*P r bl*).

F_3 Segregants of Dark Red Kidney × Red Kidney:

Fig. 26. Raisin black/light pinkish cinnamon ($P \frac{R}{r} Rk\ Bl$).
Fig. 27. Oxblood red/testaceous ($P \frac{R}{r} rk\ bl$).
Fig. 28. Black (*P R Bl*).
Fig. 29. Indian purple (*P R Bl*).
Fig. 30. Violet carmine (*P R Bl*).

F_3 Segregants of China Red × Mexican Red:

Fig. 31. Victoria lake/testaceous, with black hilum ring.
Fig. 32. Oxblood red/testaceous, with black hilum ring.

Parental Varieties:

Fig. 33. White Kidney 4516.
Fig. 34. Red Eye 4387.

F_2 Segregant of Red Eye × Buff:

Fig. 35. Buff eye.

(*All natural size.*)

PLATE 2

F_3 SEGREGANTS OF CHINA RED × MEXICAN RED:

Fig. 36. Pompeian red/testaceous.
Fig. 37. Victoria lake, with black hilum ring.
Fig. 38. Oxblood red, with orange hilum ring.
Fig. 39. Oxblood red, with no hilum ring.
Fig. 40. Pompeian red, with black hilum ring.
Fig. 41. Victoria lake, with no hilum ring.
Fig. 42. Perilla purple, with black hilum ring
Fig. 43. Oxblood red, with black hilum ring.
Fig. 44. Pompeian red, with orange hilum ring
Fig. 45. Ocher red, with orange hilum ring.

F_2 SEGREGANTS OF CHINA RED × GENEVA RED KIDNEY:

Fig. 46. Corinthian purple
Fig. 47. Liver brown.
Fig. 48. Chestnut brown.
Fig. 49. Hay's russet.
Fig. 50. Deep Corinthian red.
Fig. 51. Oxblood red
Fig. 52. Vinaceous fawn.
Fig. 53. Testaceous

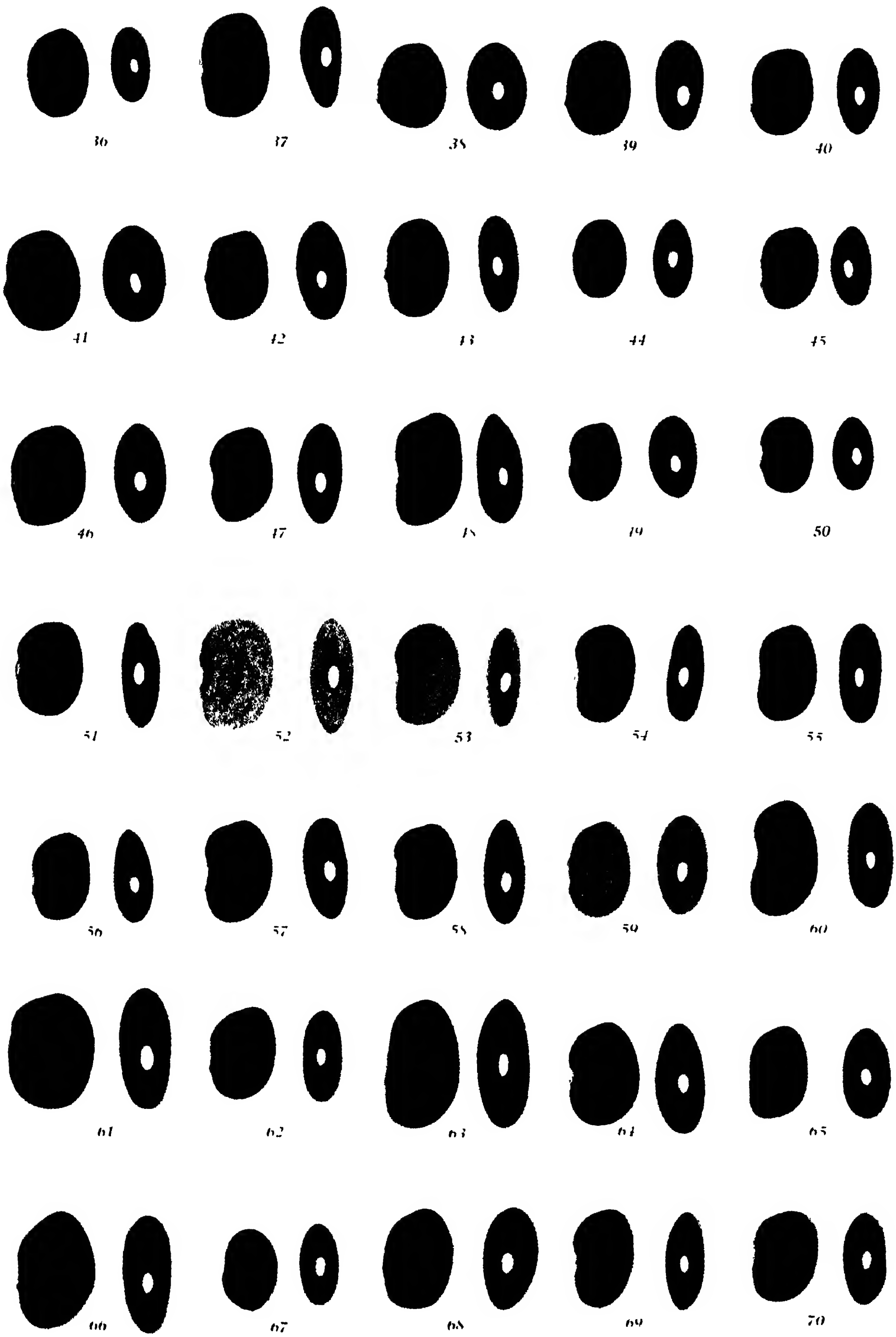
F_3 SEGREGANTS OF MEXICAN RED × RED KIDNEY:

Fig. 54. Black
Fig. 55. Victoria lake
Fig. 56. Chocolate.
Fig. 57. Oxblood red
Fig. 58. Vandyke red
Fig. 59. Testaceous

F_3 SEGREGANTS OF MEXICAN RED × DARK RED KIDNEY:

Fig. 60. Indian purple/testaceous
Fig. 61. Perilla purple/light perilla purple
Fig. 62. Oxblood red/Pompeian red
Fig. 63. Indian purple, with black hilum ring
Fig. 64. Indian purple, with no hilum ring
Fig. 65. Perilla purple, with black hilum ring
Fig. 66. Oxblood red, with black hilum ring
Fig. 67. Oxblood red, with orange hilum ring
Fig. 68. Oxblood red, with no hilum ring
Fig. 69. Acajou red, with black hilum ring.
Fig. 70. Acajou red, with orange hilum ring.

(*All natural size.*)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SOME MOSAIC DISEASES OF PRUNUS SPECIES[1]

H. EARL THOMAS[2] AND T. E. RAWLINS[3]

IN THE ABSENCE of a visible fungus or bacterial pathogene, the diagnosis of a plant disease depends in a large measure upon the recognition of symptoms. This observation is particularly applicable in the study of diseases of deciduous fruit trees in California where there is found a relatively greater proportion of virus and nontransmissible diseases than is the case in more humid areas. It has seemed desirable, therefore, to assemble and to study, under comparable conditions, some of the prevalent virus diseases of deciduous fruit trees and to improve the criteria for distinguishing these from nontransmissible diseases such as exanthema and little-leaf, or rosette, the latter of which may cause symptoms on grape leaves strongly suggestive of a mosaic disease.

It is a rather remarkable fact that none of the several well-known virus diseases of stone fruits in eastern United States, namely, peach yellows, peach rosette, little peach, and phony peach, have been found in California. On the other hand, there are a considerable number of virus diseases of these plants in California, few or none of which seem to be established in the eastern states. Among the latter group, only cherry buckskin (*8*)[4] and peach mosaic (*3*, *6*) have been studied in any detail.

The earlier plan of this work, begun in 1932, involved the collection of those virus-type diseases that were found on deciduous fruit trees from which the more important were to be selected for further study. As the collections continued, however, and certain apparent interrelations between some of the diseases appeared, it became more and more

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[4] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

difficult to single out one disease to the exclusion of others. As a consequence, collection and cross-inoculation with a number of these diseases have been continued to the present, and some of the seemingly minor diseases are set forth here as far as information is available, along with several which appear to be of considerable importance in the orchards of central California. This paper is limited to diseases of the mosaic type, using the term "mosaic" in a rather broad sense.

ALMOND MOSAIC DISEASES

Speckling and mottling of foliage of almond, *Prunus communis,* first called to notice by Gilbert L. Stout, is very prevalent in orchards of the

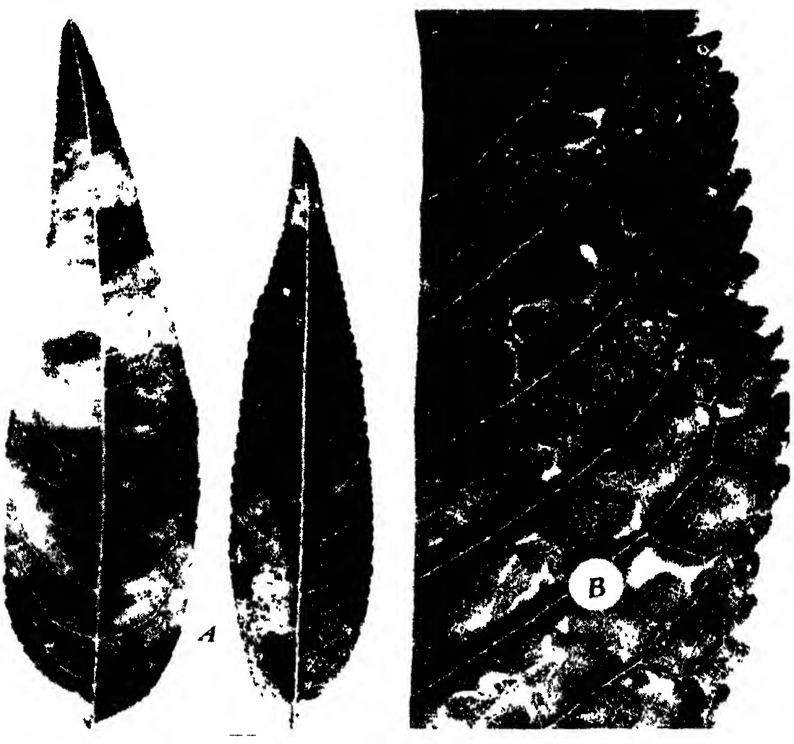

Fig. 1.—*A*, The calico disease of almond; *B*, part of a leaf from mazzard-cherry seedling inoculated with the almond-calico virus.

northern Sacramento Valley. It is not clear at present how many diseases are involved. While the symptoms on individual leaves are often conspicuous, the damage to the tree as a whole does not seem to be great (see also section on "Peach Mosaic Diseases").

A common symptom is a small, pale green to white, more or less star-shaped spot in the leaf blade. At other times large chlorotic blotches or

bands are seen, usually with little or no distortion. Material representing the latter symptoms, collected by Stout at Chico and designated by him as "calico" (fig. 1, *A*), was used to inoculate almond and peach. On small potted almond trees in the greenhouse, symptoms appeared as early as 4 months after inoculation, while on trees eight to ten years old symptoms were not seen on previously healthy branches until 19 months after inoculation.

On seedling peach trees inoculated with the affected almond material, a few leaves eventually developed large, pale-yellow blotches without pattern, the symptoms apparently becoming intensified as the leaf matured. These symptoms closely resembled some of those produced on peach out of doors by the cherry-mosaic-1 virus.

Inoculations with the above almond material to small sweet-cherry seedlings resulted in mild to striking symptoms within 7 weeks, varying considerably from plant to plant and in some leaves resembling symptoms which are found on cherry in the orchard (fig. 1, *B*).

APRICOT MOSAIC DISEASES

Mild but definite mosaic symptoms on apricot, *Prunus Armeniaca,* have been found on a few trees in each of three orchards in Solano and Yolo counties. Buds from one of these trees were used to transmit the disease (fig. 2) to seedling apricot trees. Trees budded in October exhibited symptoms near the points of inoculation the following May, but about 18 months were required for symptoms to appear in all parts of trees eight to ten years old. The symptoms in one such inoculated tree have been distinctly more severe than those seen in the original orchard trees. No certain symptoms have been seen thus far in seedling peach trees up to two and one-half years from the time of inoculation with this disease. In some inoculated apricot trees, but not all, a distinct shortening of nodes was visible the second or third year after inoculation. At present this disease is of interest chiefly because of the similarity of symptoms to those of a disease of peach and apricot treated later in this paper (p. 633).

CHERRY MOSAIC DISEASES

A considerable range of chlorotic symptoms has been seen on sweet cherry, *Prunus avium.* The infectious nature of some of these chloroses, however, is in considerable doubt. In fact, some of the common types of chlorosis which simulate virus symptoms, seem to be definitely not transmissible. Some of these have been grafted on apparently healthy sweet-cherry trees and the inoculated trees have remained symptomless for at

least two or three years, even the affected scions failing to retain the symptoms. Such a type shown in figure 3, *A* seems to be the result of unfavorable soil.

On the other hand, at least two types of mosaic of the sweet cherry are transmissible by grafting. One of these diseases, evidenced by chlorotic

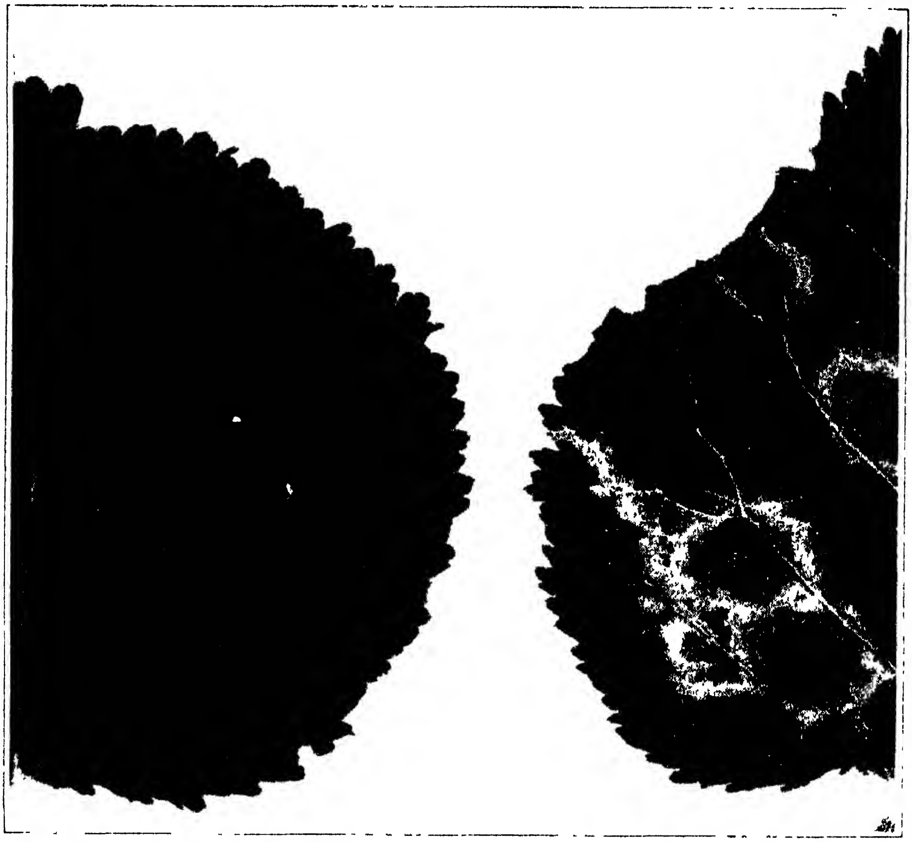

Fig. 2.—A mosaic of apricot.

blotches, lines, or rings (fig. 3, *B*), seems to be more apparent in the foliage of mazzard (*Prunus avium*) rootstocks than in the common top varieties of sweet cherries and altogether comparatively mild in effect upon the more common orchard varieties. Plants inoculated with this disease in October developed symptoms the following spring.

Symptoms somewhat similar to these were seen on a few trees of the varieties Windsor and Yellow Spanish in Wayne County, New York, in 1931. In a preliminary attempt to transmit the disease from Yellow Spanish to Black Tartarian in the greenhouse at Ithaca, New York, symptoms appeared the following spring but did not persist, and the

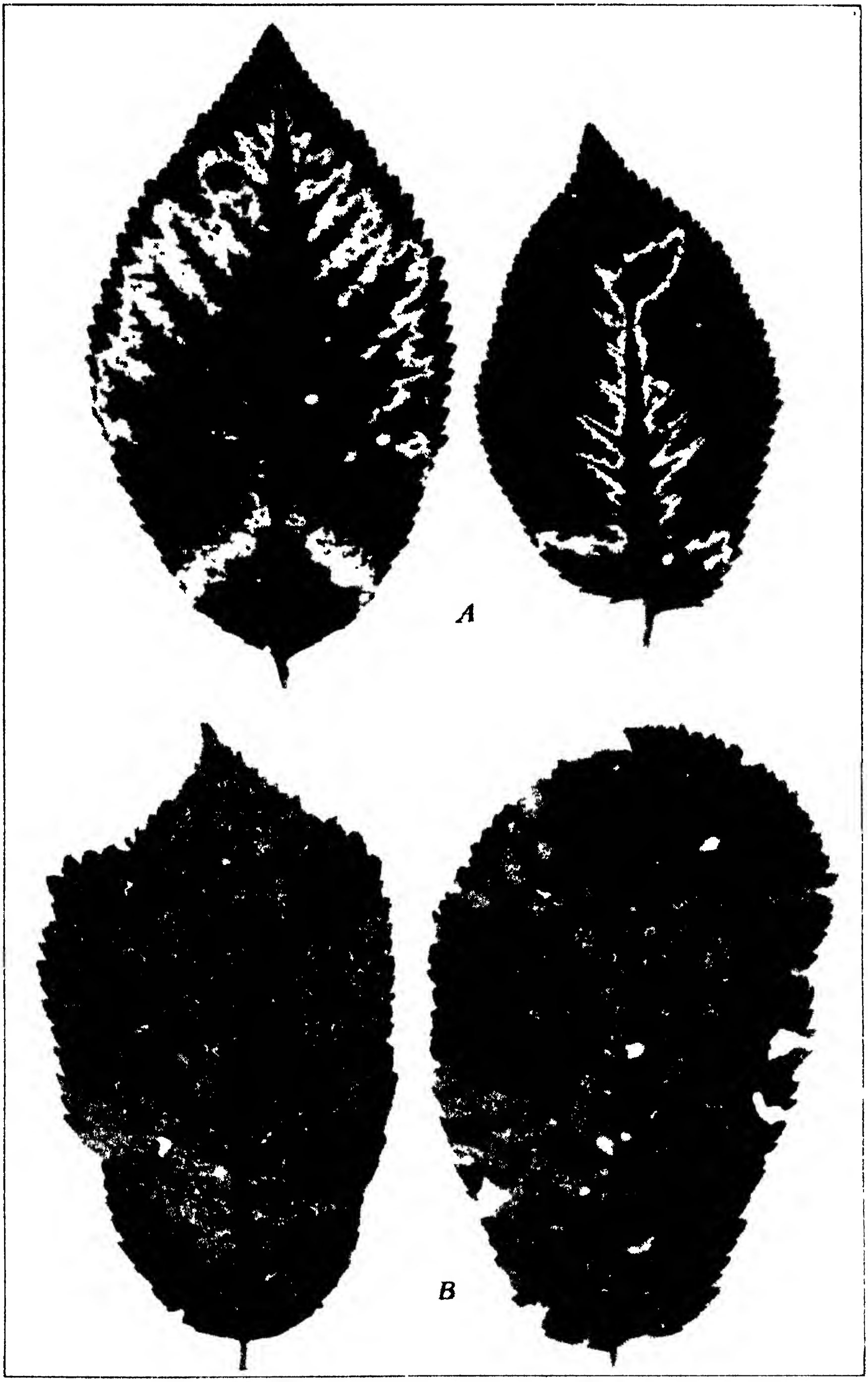

Fig. 3.—*A*, Noninfectious condition in sweet cherry apparently related to unfavorable soil; *B*, a mosaic of mazzard-cherry foliage.

results are considered inconclusive. C. E. F. Guterman very kindly noted the results of this experiment.

Another type of mosaic (fig. 4) is somewhat similar to "mottle leaf" reported from Oregon (*14*).[5] It is apparently distinct, however, from the disease treated under that name by E. L. Reeves in Washington

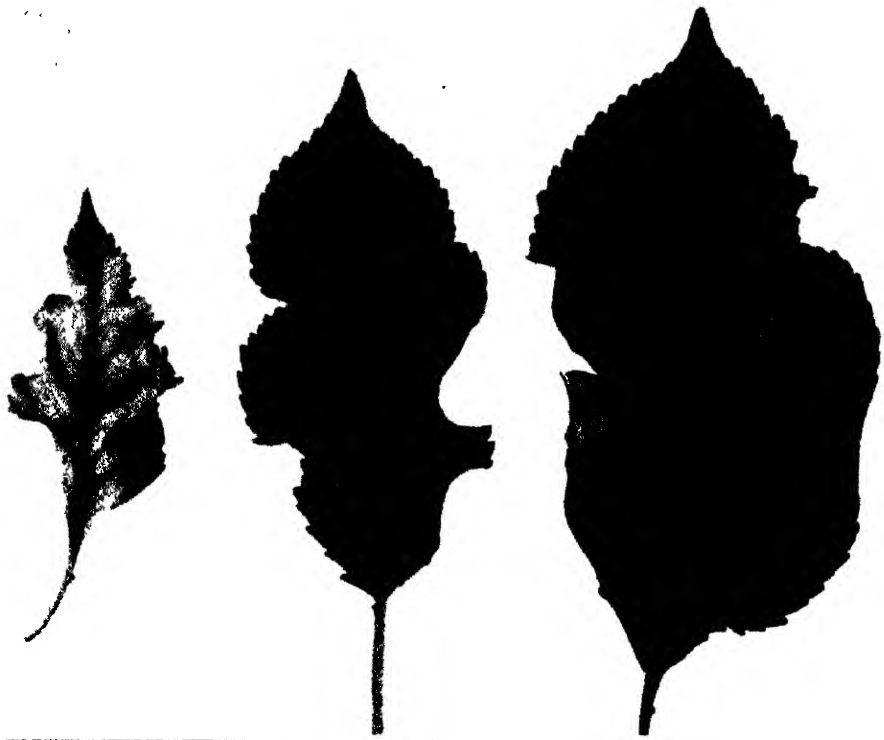

Fig. 4.—Early-season symptoms of cherry mosaic 1 on leaves of Napoleon variety; natural infection.

(*10*) ; Reeves has pointed out in one orchard in Napa County, California, the type of disease he has under study in Washington.

Since the name "mottle-leaf" has seemingly been applied to a disease distinct from that under consideration here, and since it is already in general use in this state to designate a nontransmissible disease of citrus, the disease treated in the following paragraphs will be designated "cherry mosaic 1." This disease has been seen on Black Tartarian, Chapman, Napoleon (Royal Ann), and Republican (Black Republican), and

[5] H. R. McLarty, in a paper entitled "Cherry Mottle Leaf," presented before the Northwest Association of Horticulturists, Entomologists, and Plant Pathologists at Kelowna, B. C., July 17–19, 1935, expressed the opinion that the disease studied by him in British Columbia was the same as that described by Zeller in Oregon (*14*). In this paper McLarty presented evidence of the infectious nature of the disease.

a variety known locally as "Long Stem Bing," and has been observed in Butte, Napa, Riverside, Solano, Sonoma, and Sutter counties. The variety Napoleon seems to be somewhat more susceptible than the others mentioned (as was the case in Oregon).

The early-season symptoms of cherry mosaic 1 are small to large chlorotic blotches in the blade of the young leaf followed by distortion as the green portions of the leaf continue to expand (fig. 4). The chlorotic areas of such leaves often drop out, and many of the leaves fall by midsummer. In the latter part of the season a milder mottling of leaves with little or no distortion is often seen on affected trees. Rather consistently associated with this disease in late stages is the appearance of compact tufts of small and sometimes distorted leaves from latent buds on large branches of older trees. In advanced stages of the disease, the fruit is scant and in some varieties tends to be somewhat misshapen.

Potted trees of the Napoleon variety and seedlings inoculated by affected scions or buds in May and June developed symptoms early the following spring. Observations thus far, however, indicate that in general the spread of this disease is rather slow in orchards. For example, in one orchard of Black Tartarian and Napoleon, 5 of 85 trees were noted as affected in June, 1935, and only 6 of the 85 trees were unmistakably affected in May, 1938. An apparent exception to this general impression was encountered in one orchard of 80 acres of Republican and Napoleon where the grower had seen only 5 or 6 affected trees in the entire orchard during the five years preceding 1937. In a portion of this orchard, 5 of 100 trees examined in May, 1928 (mostly Napoleon) were affected and 8 others appeared to be so.

Potted peach trees inoculated by inarching or budding with affected cherry material have shown a considerable range of symptoms, but mild for the most part. These symptoms include in some cases distinct chlorotic blotches in the expanding leaf resembling the symptoms on young cherry leaves. On other plants mild to rather strong chlorosis is produced, typically without any definite pattern, and with little or no distortion. Preliminary tests indicate that cherry mosaic 1 is transmissible by inarching to almond and *Prunus Mahaleb,* producing rather mild symptoms on these plants.

A disease of sour cherry, *Prunus Cerasus,* which causes a mottling of the leaves, is common both in New York orchards and in the comparatively few trees that have been examined in California. These trees are of interest here chiefly for the reason that they are sometimes used as stocks for sweet cherry.

The symptoms of the disease of the sour cherry in the two states are

sufficiently similar to suggest that the cause is, in some cases, the same. Usually the affected leaf develops pale-green to yellow blotches with some distortion. Occasionally definite chlorotic bands and rings appear. A symptom which seems to be a part of the same complex in both states consists in small rings clearly apparent by reflected light but invisible or nearly so by transmitted light and without appreciable chlorosis.

Sweet-cherry scions and buds grafted on affected sour-cherry trees (morello type) in California have not shown any symptoms clearly related to the sour-cherry disease. Peach trees inoculated with this disease both at Ithaca, New York, and at Berkeley, California, developed mild mottling of leaves, and at Berkeley, strong vein swelling when the plants were kept in the greenhouse.

Another disease occasionally seen in leaves of mahaleb rootstocks, on some of which sweet cherries are growing, produces symptoms usually consisting in rather broad chlorotic bands. This type of mosaic was readily transmitted by grafting to healthy mahaleb trees, the symptoms appearing within 4½ months after inoculation. However, no specific effect of such diseased stocks is apparent on the sweet-cherry trees grown on them for several years.

PLUM AND PRUNE MOSAIC DISEASES

At least two types of mosaic on the Japanese plum, *Prunus salicina*, and two on prune, or European plum, *P. domestica*, have been under observation.

One of the mosaics on the Japanese plum has thus far been recognized only in a single planting of the variety Santa Rosa (*Prunus salicina*) in Vacaville, Solano County. Symptoms are mild, consisting in pale-green blotches, lines, and rings in the leaf blade (fig. 5, *C*). There is no evidence as yet, after five years from the first observation, that this disease is causing appreciable reduction in vigor of the trees. The disease is transmitted by buds to peach seedlings in which the symptoms in the greenhouse consist in mild mottling without any very definite pattern. This disease will be referred to later (p. 642) as the "Vacaville plum mosaic." It is similar to a mosaic of plum, transmissible to peach, which has been reported from Kentucky (*13*).

A second disease or group of similar diseases has been found on the Santa Rosa plum, and more recently on other varieties, in Eldorado, Mendocino, Placer, Santa Cruz, Solano, and Yolo counties. The typical symptoms on the Santa Rosa are rather small, completely chlorotic spots more numerous toward the distal end of the leaf blade (fig. 5, *A*). The Duarte variety in one orchard and an unnamed variety in another dis-

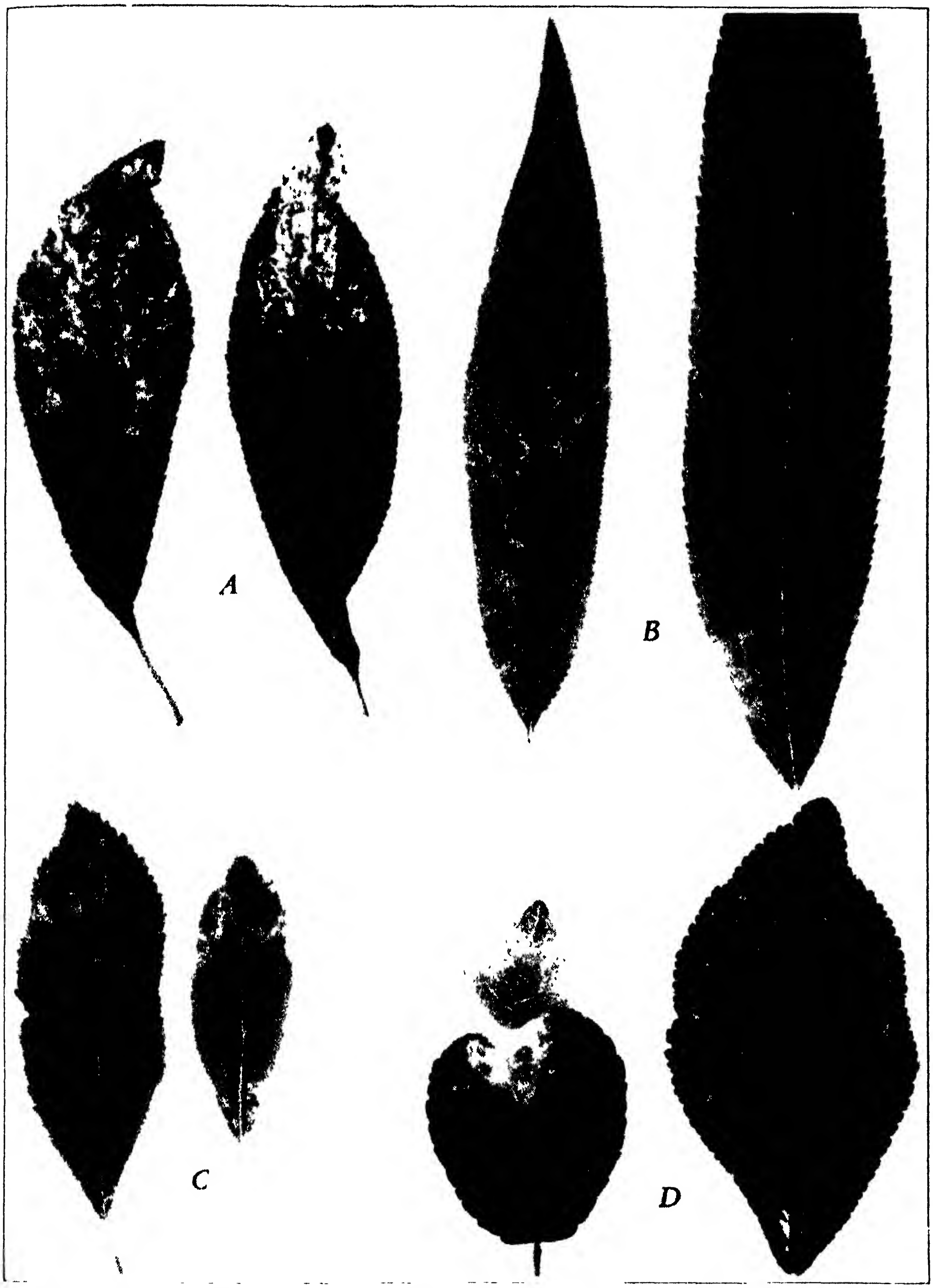

Fig. 5.—*A*, A mosaic on Santa Rosa variety of Japanese plum, natural infection at Aptos; *B*, Standard-prune-mosaic symptoms on seedling peach leaves; *C*, Vacaville plum mosaic on Santa Rosa variety; *D*, mosaic of Standard prune, natural infection at Live Oak.

played symptoms resembling those on Santa Rosa while the myrobalan (*Prunus cerasifera*) shoots from the rootstocks of affected trees in these orchards bore strong lines, bands, and vein clearing. The varieties Inca, Kelsey, and Sharpe's Pearl were also seen with symptoms similar to those of Santa Rosa, while the Del Norte in one orchard exhibited vein clearing only.

The latter disease of the Santa Rosa plum was transmitted by grafts to healthy Santa Rosa trees, and the myrobalan from one of the orchards mentioned was used successfully in transmitting the disease to healthy myrobalan plants.

Observations thus far indicate that this disease impairs the vigor of the trees appreciably but probably not greatly, at least in any short period of years.

A mosaic of Standard prune was noticed by C. E. Scott and H. H. Thornberry in October, 1935, in an orchard at Live Oak, California. Not less than 80 or 90 per cent of the trees in this orchard are affected, and there seems to be a slow but eventually considerable decline in vigor of affected trees. A small lot of trees of this variety purchased from a local nursery in 1937 all appeared to be affected by the same disease.

Typical symptoms on the prune are few to many small chlorotic spots, often more numerous in an area toward the tip of the leaf and coalescing to cause distortion and dropping out of parts of the leaf blade. (fig. 5, *D*). Unlike most of the diseases under consideration in this paper, this disease is more apparent in midseason than in early spring or in the greenhouse than out of doors, which indicates that a relatively high temperature is favorable for its development.

The prune orchard at Live Oak is interplanted with J. H. Hale peach trees, and the prune trees are growing on peach roots. No symptoms have been seen on the J. H. Hale trees that seem to be clearly related to the prune disease, but shoots from the peach rootstocks of the prune trees occasionally exhibit a rather mild mottling of the leaves. Also when peach seedlings were inarched in the greenhouse with affected Standard prune in June, 1936, the peach leaves developed a swelling of the veins by November of that year, and the following spring, a mottling varying from mild and rather indefinite to very striking lines and rings (fig. 5, *B*).

A disease of Sugar prune, noticed by W. D. Butler and M. R. Harris in an orchard near Napa, California, is similar to that on Standard prunes in appearance, with the difference that the basal part of the leaf is more often affected, and there is more tendency for the affected tissues to drop out. When healthy Sugar prune was inoculated in the green-

house by inarching with affected Standard prune (from Live Oak), only doubtful symptoms were seen on the Sugar prune the following year. On orchard trees of Sugar prune inoculated with affected buds of the same variety in July, 1936, symptoms were seen in August, 1938, only on shoots which grew from the affected buds. Likewise, inoculations of Agen (French) prune, President plum, peach seedlings, and *Prunus subcordata* have failed thus far to produce symptoms except that the peach leaves exhibited a marked vein swelling the year after inoculation.

The diseases of Standard and Sugar prunes are distinct in appearance from the virus disease of Italian Prune (Fellenberg) found in western New York (*11*).

PEACH MOSAIC DISEASES

Mosaic diseases of the peach, *Prunus Persica,* are of particular interest at this time because of the destructive disease (*1, 6*) found in several southwestern states, including southern California, and designated heretofore merely as "peach mosaic." This disease will be referred to when necessary in the present paper as the "Texas peach mosaic," since it was first found in that state. A distinct disease designated as "asteroid spot" has also been found on peaches in southern California (*4*).

In addition to the several mosaic diseases already described in this paper which may be transmitted by inoculation to the peach, at least[6] one distinct mosaic-type disease has been found occurring naturally on peaches in central California. This disease is of especial interest in several ways and is discussed in some detail. It is known at present in only two orchards in the vicinity of Winters, California, and will be referred to as the "Winters peach mosaic."

INVESTIGATIONS OF WINTERS PEACH MOSAIC

Symptoms.—The most conspicuous symptoms of Winters peach mosaic are in the leaves and leaf buds and these are most readily seen at the beginning of the growing season. Pale-green to pale-yellow, oblong, feather-edged blotches appear along the midvein or larger lateral veins before the leaves are fully expanded, which result in distortion of the lamina and often dropping out of the chlorotic parts (fig. 6, *A*). Later in the season some leaves are seen with milder symptoms of the sort shown in figure 6, *B*, and rarely, a leaf is seen with definite chlorotic lines and rings.

[6] Mild mosaic mottling on a flowering-peach variety at Santa Clara and another near Fairfield on a fruiting variety (first noticed by L. C. Cochran) have not been studied in any detail.

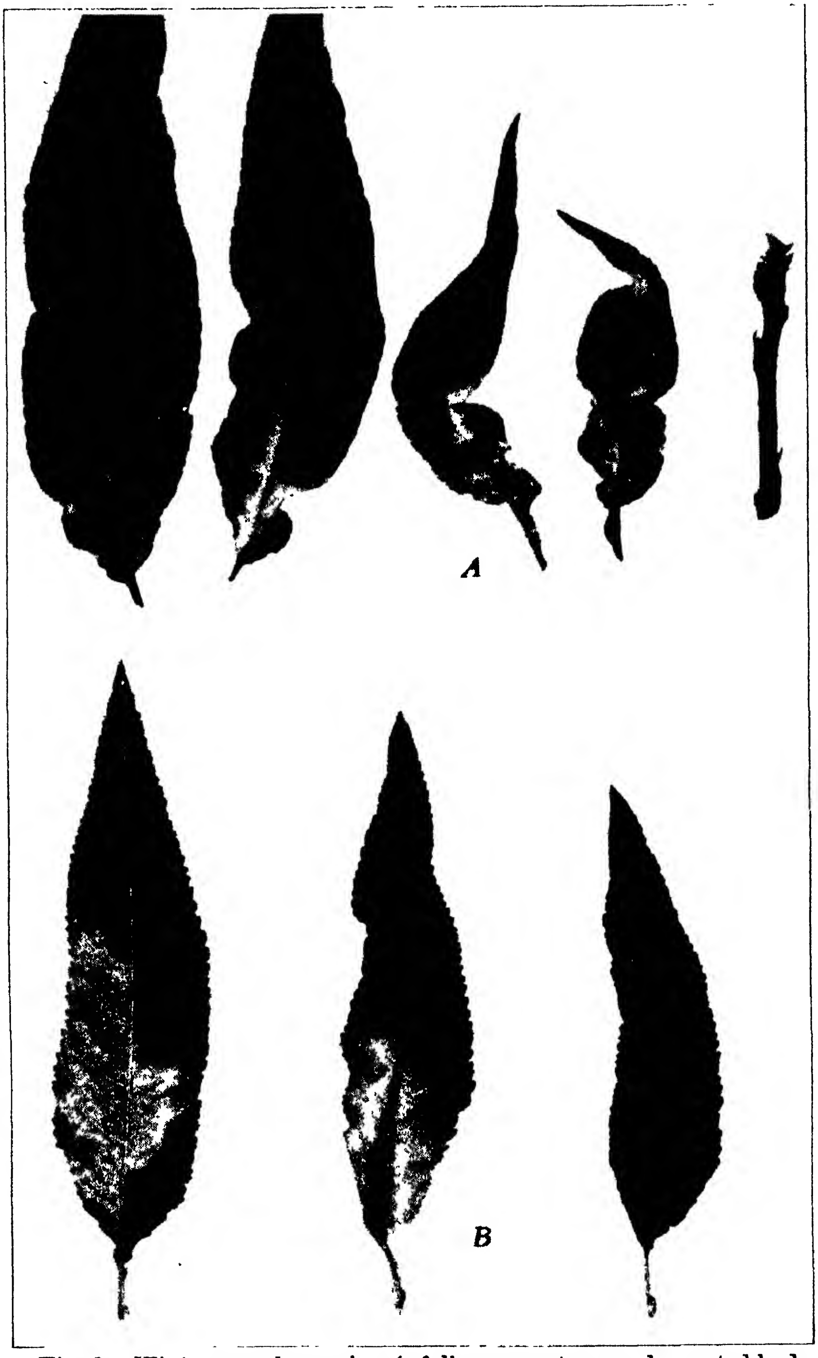

Fig. 6.—Winters peach mosaic: *A*, foliage symptoms and arrested buds of seedling peach; *B*, foliage symptoms on leaves of Elberta peach in the field in August.

On severely affected branches, the leaf buds often push out pale leaf tips for a few millimeters and then remain practically unchanged for a period of several weeks (fig. 6, *A*). Eventually these branches die back or a few buds produce compact clusters of small narrow leaves, often somewhat curved laterally, and usually with no conspicuous mottling. A considerable number of affected peach trees of both Elberta and Muir have died in the two orchards under consideration, but there is some evidence that unfavorable soil conditions have contributed to this loss.

When potted peach trees are inoculated in the greenhouse, most of the early-season symptoms found in the orchard are observable. The symptoms on these small trees, mostly seedlings, are on the whole more severe than those in the orchard and seem to be more severe at the onset of disease than later, on smaller trees than on larger trees, and when a scion is used as inoculum than when a bud is used. When a potted tree is cut back to within a few inches of the soil level and inoculated by grafting with a diseased scion, the tree seldom makes appreciable growth afterward, usually dying within a few weeks or months. When trees in the greenhouse are inoculated by budding after considerable foliage has developed, the youngest leaves at the time symptoms appear resemble those in the orchard in early spring; leaves somewhat older develop similar but small chlorotic areas; still older leaves develop numerous minute chlorotic spots in the leaf blade without distortion; while the oldest leaves exhibit no symptoms.

No symptoms in the flowers have been noted. Only a few flowers of affected peaches, other than Elberta and Muir, have been seen.

Fruit development varies from a fair crop to a few or none on affected branches or trees, more or less in proportion to the stage of development of the disease. Many Elberta fruits on affected trees in 1937 grew for a time, but remained slender and finally withered and dropped. This may have been due to a combination of low vigor and poor pollination. Fruits on affected Muir peach trees sometimes develop appreciable irregularities in shape (fig. 7) but not so pronounced as in the case of the Texas peach mosaic (*6*) in some varieties.

Experiments with Winters peach mosaic on other *Prunus* species have been made. On apricot, affected leaves in the orchards and in the greenhouse usually develop rather large chlorotic areas more nearly circular than on peach and rather frequently exhibit clearly defined chlorotic lines and rings (fig. 8, *A*). The affected areas often drop out but may persist for several months. There is some tendency also in the apricot for the buds to be arrested in early stages. On the whole, the Winters peach mosaic is distinctly milder on apricot than on peach.

No flower or fruit symptoms have been recognized on apricots.

The Winters peach mosaic on apricot is distinguishable from the disease mentioned earlier in this paper (p. 625) by greater severity of symptoms and by the failure of the latter to affect peach.

Only a few almond trees that appear to be affected by Winters peach mosaic have been found in orchards, and the identity of the disease on

Fig. 7.—Dwarfing and malformation of Muir peach fruits from trees naturally infected by Winters peach mosaic.

these is in some doubt. In these cases, part of the leaf blade or all of it is strikingly chlorotic in early spring (fig. 8, *B*), and later, compact tufts of leaves appear somewhat similar to those sometimes seen on peach, but larger.

The fruits of these trees are irregular in shape, but somewhat similar fruits are rather common in central California on trees not always displaying any other symptoms.

Symptoms on Texas and seedling almond trees inoculated in the greenhouse with the Winters-peach-mosaic virus from peach or apricot vary from mild to severe, consisting in chlorotic spots on young leaves and tufting of older leaves which are often undulant and laterally curved as on some of the trees in the orchard. There is a tendency here also for arresting of the buds (fig. 9), which may die afterward or develop slowly into distinct rosettes.

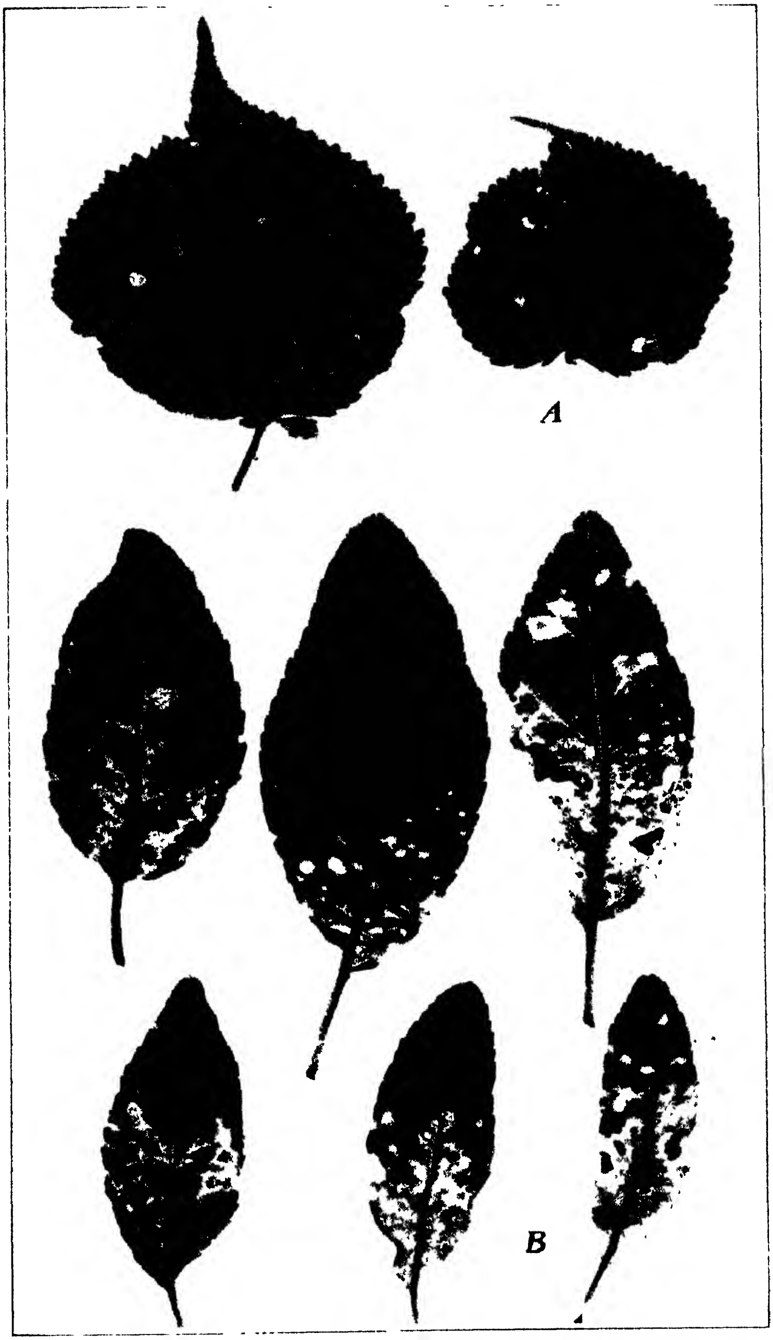

Fig. 8.—*A*, Winters peach mosaic on seedling apricot leaves in the greenhouse, the leaf at right shows symptoms nearest those found in the orchard. *B*, A mosaic on Texas variety of almond, natural infection, early-season symptoms.

Fig. 9.—Winters peach mosaic on almond seedlings produced by inoculation in greenhouse. The larger plant was inoculated at the point indicated by the arrow on June 15, 1937, and photographed on February 23, 1938. Only the buds beyond the point of inoculation show symptoms. The plant at left is affected throughout and is in a more advanced stage of growth.

Symptoms of the Winters peach mosaic on plants other than peach, almond, and apricot have been seen only on plants inoculated and kept in the greenhouse. Some will be mentioned in the following sections.

Sap and Temporary Inarching Inoculations with Peaches.—A number of experiments were made in which affected peach leaves were ground

in a mortar in water alone or in water plus various materials, such as agar, gelatin, gum acacia, and the natural gums of several stone fruits. The sap was then rubbed on young peach leaves using carborundum (*9*) as an abrasive. The results to date, involving 61 trees, have been uniformly negative.

In a single experiment 5 healthy peach seedlings were inarched on diseased plants and bound with rubber budding tape in the same way that other inoculations were made except that no wax was used. The inoculated plants were detached after 79 hours, and the wounds were covered with a proprietary grafting tape. At the end of 12 weeks no infection was apparent on these plants. For a peach mosaic from Colorado, Kunkel (*7*) has recently shown that infected buds must be left in inoculated plants for a minimum of 2 days in order to produce infection.

Grafting Inoculations with Peaches.—The Winters peach mosaic was transmitted readily from peach to peach in the greenhouse by buds, scions, or inarches, not often failing even when the inoculum did not survive, as was frequently the case with diseased buds. The minimum incubation period observed was slightly less than 4 weeks.

Grafting Inoculations with Related Species.—Although it does not necessarily follow that a susceptible plant will become inoculated under natural conditions, for several reasons it is desirable to know, as far as possible, the range of susceptible plants, particularly for a disease likely to be dealt with by eradication. Accordingly, inoculations on a small scale were made on a considerable number of species more or less related to peach. In distantly related plants, and in most of the others, inarching was used as the method of grafting. With this method, graft unions can be obtained which would not be possible with buds and scions. Presumably also this method exposes the test plant to a greater mass of inoculum. It is not possible in all cases, however, to determine whether a union has been made even when both plants produce abundant callus. Neither has an actual growth union been shown beyond question to be essential for the transmission of this disease. At any rate, negative results are held as doubtful unless a definite union was established.

Infection with Winters peach mosaic was obtained with about equal facility from peach to apricot and apricot to peach as from peach to peach. Infections have been obtained from both peach and apricot to almond but with more difficulty. Symptoms in the almond were slow in developing, in some cases severe (fig. 9), but frequently mild in the end; a few failed to develop any marked symptoms even after the virus had been recovered from them by inoculation to peach or other plant.

Symptoms sometimes appear above and below the point of inoculation

at about the same time, but more often they appear first above. In several almonds inoculated with Winters-peach-mosaic virus early in 1937, strong symptoms appeared in the spring of 1938 in all leaves distal to the point of inoculation but not elsewhere on the plants during 1938 (fig. 9). However, the virus was demonstrated in 7 of 10 scions taken from seemingly healthy parts of such plants on July 11, 1938.

Although several almond trees, and one in particular, have been seen in the orchard which seemed to be affected by the same disease, several attempts to produce the typical disease in peach or apricot by inoculation from such almonds have yielded inconclusive results. When scions from the most severely affected almond tree found in the orchard (fig. 8, *B*) were grafted on peach or almond in the greenhouse in March, 1937, a few leaves on the stocks of both peach and almond bore symptoms a few weeks later which resembled those of the typical disease, but the symptoms did not persist, and some of these plants appeared to be healthy as late as July, 1938.

Inoculation of plants of the desert peach, *Prunus Andersonii*, growing on their own roots has thus far failed to produce symptoms even when they made good graft unions and were left exposed to the inoculum for several months. But 2 of several scions taken from that part of an inoculated plant distal to the point of inoculation and grafted on healthy peach seedlings in 1937 developed very pronounced symptoms in the spring of 1938 (fig. 10) consisting in compact tufts of small leaves and suppression of shoot growth.

Of 7 cherry trees (*Prunus avium*) inoculated in March and June, 1937, one began to develop dwarfed and distorted leaves 2 months after inoculation and in the spring of 1938 was severely affected with mostly small pale leaves slow in appearing and with no shoot growth. The virus was recovered from this plant by inoculation to peach. Doubtful symptoms were seen on 2 of the other cherry trees, but attempts to recover the virus from them have not thus far been successful.

Small-scale attempts to infect *Prunus cerasifera* (myrobalan), *P. domestica* (Sugar prune and Tragedy plum), *P. ilicifolia*, and *P. lusitanica* seem to have failed as have attempts to recover the virus from myrobalan and Sugar prune.

Prunus Mume was infected by inoculation from apricot with symptoms resembling those of the apricot.

Inoculation of cultivated strawberry and red raspberry failed to produce symptoms. No attempt was made to recover the virus.

The single-flowered type of *Kerria japonica* inoculated in 1937 developed a rather indefinite mottling of the foliage in 1938 hardly suffi-

cient to remove the plant from the symptomless-carrier class, but the virus was recovered readily from this plant by inoculation to peach seedlings.

This virus was transmitted with some difficulty to Gloire des Rosomanes (Ragged Robin) rose (2 of 6 inoculated plants) but with very

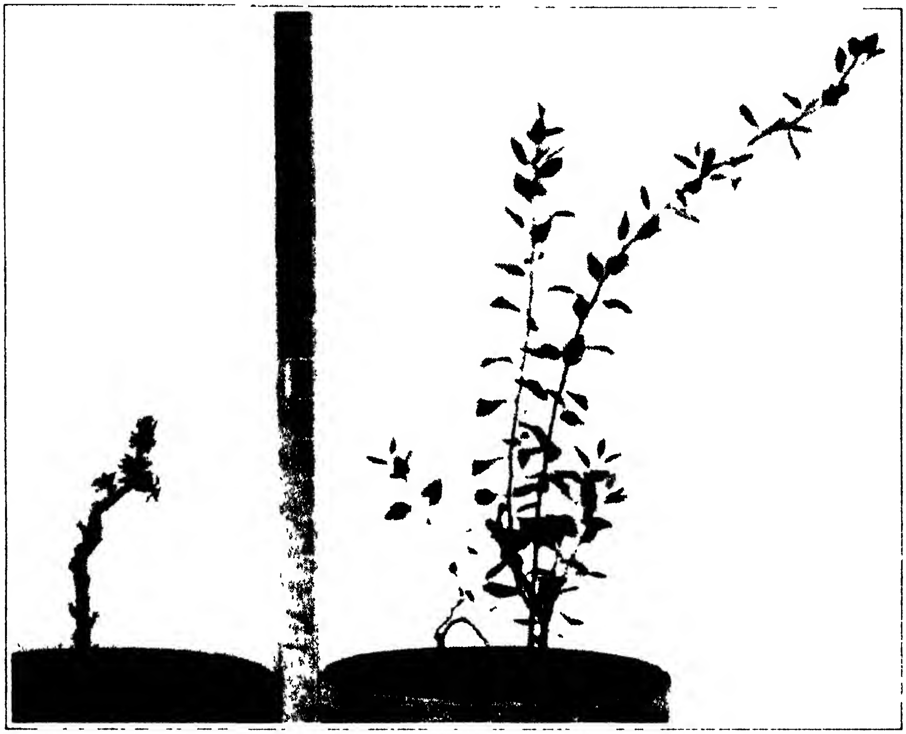

Fig. 10.—At left, Winters peach mosaic on desert peach, *Prunus Andersonii*, grafted on peach seedling root. At right, the *P. Andersonii* plant from which the affected scion at left was taken has no symptoms.

pronounced symptoms on those plants that became infected. A characteristic noted on plants of other species affected by this disease but more conspicuous on the rose, is the tendency for extremely chlorotic areas to develop considerable green pigment with advancing age of the leaf. Some details of symptoms are treated in the accompanying paper on mosaic diseases of the rose (*12*).

Attempts to Induce Immunity.—When it became apparent that the desert peach, *Prunus Andersonii*, did not develop symptoms within several months after inoculation with Winters-peach-mosaic virus, and that the virus did not seem to pass readily through this plant, shoots of affected cultivated peach trees were inarched in 1937 on plants of *P. Andersonii* and later detached so that they were entirely supported by

the latter. Six such combinations survived the following winter of 1937–38. The peach scions in 3 of these cases died the following spring without producing new leaves. Two others exhibited symptoms, at least one of them as severe as with peach on peach root. A single scion remained healthy throughout 1938 but possibly for the reason that it had not yet been invaded by the virus at the time it was detached from the parent plant. None of the *P. Andersonii* plants in this group, however, bore symptoms up to December, 1938, nor have any others of this species while growing on their own roots, although infection has been obtained in *P. Andersonii* growing on peach roots. This may or may not be comparable to the interesting case of apparent recovery of diseased scions when grown on resistant or immune stocks which has been reported for the bunch disease of pecan (*5*).

As a result of cross-infection experiments and the types of symptoms displayed, the following diseases were selected to use in attempts to immunize peach seedlings against the Winters peach mosaic: almond calico, cherry mosaic 1, Vacaville plum mosaic, and the Standard prune mosaic. Twelve, 14, 10, and 10 seedling peach trees, respectively, were inoculated with these diseases by scions or by inarching in October, 1937, and after overwintering were inoculated by buds carrying the Winters-peach-mosaic virus inserted near the ground line in March, 1938. While this experiment is not entirely concluded at the time of writing, 6, 6, 7, and 5 plants, respectively, in the 4 groups have developed symptoms of the Winters peach mosaic, and no clear reduction in severity has been noted.

DISCUSSION

A determination of the severity of the mosaic diseases discussed in this paper will involve not only the inoculation of varieties and species related to those in which the diseases occur naturally but also the growing of affected trees under orchard conditions known to be relatively favorable for the growth of the particular trees under consideration. In many cases, moreover, the trees must be kept under observation for several years, for a slow decline may easily pass unnoticed but eventually cause more loss than a disease which kills the trees in a single season.

The relation of these diseases to each other and to those in other states and countries is in some cases uncertain. The confused state of the knowledge of such diseases is illustrated by recent papers from central Europe (*2*). A final determination of the relation between similar diseases in different localities would often require the comparison of infected plants side by side grown under the same conditions. This is not feasible with anything short of equipment for complete isolation.

Symptoms on the several species and the results of cross-inoculations indicate that almond calico, cherry mosaic 1, and the Winters peach mosaic may be related, but certain of the symptoms and the results of an immunization experiment seem to show that they are not identical.

The control of these diseases in established orchards whenever deemed necessary will no doubt consist chiefly in the eradication of affected trees, as is the case with other virus diseases of fruit trees. In a few instances, however, there is evidence that spread of the diseases in the orchard may be less important than that in the nursery. At any rate, a complete control program must inevitably involve the use of scion wood from trees which are carefully examined at several seasons of the year for symptoms of virus diseases.

SUMMARY

One disease or more of the mosaic type have been found in central California affecting each of the following species of *Prunus: P. Armeniaca, P. avium, P. cerasifera, P. Cerasus, P. communis, P. domestica, P. Mahaleb, P. Persica, P. salicina.* These vary in effect upon the known suscepts from mild to severe. Symptoms are described and illustrated.

A mosaic of *Prunus avium*, designated as "cherry mosaic 1," is rather widely distributed in the state and is transmissible to *P. Persica* and apparently to other species.

Almond calico is transmissible to *Prunus avium* and *P. Persica* with rather strong symptoms on cherry.

At least two mosaic diseases were found in Japanese plum, *Prunus salicina.* One of these, the Vacaville plum mosaic, on the Santa Rosa variety was transmitted to peach seedlings.

Mosaic diseases of Standard and Sugar prune (*Prunus domestica*) are similar in certain symptoms but the evidence to date indicates that they are not identical. The disease of Standard prune was transmitted to peach.

A mosaic of *Prunus Persica,* known thus far only in one locality at Winters, is designated "Winters peach mosaic." It is in many respects similar to the mosaic of peaches in Texas, southern California, and elsewhere. This disease occurs naturally on peach, apricot, and probably on almond. It has been transmitted by grafting to *Prunus Andersonii, P. Armeniaca, P. avium, P. communis, P. Mume, Kerria japonica,* and *Rosa* sp. (Ragged Robin).

An attempt to immunize peach seedling trees against the Winters peach mosaic by the use of several milder mosaic viruses was not successful.

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MOSAIC DISEASES OF THE ROSE IN CALIFORNIA

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MOSAIC DISEASES OF THE ROSE IN CALIFORNIA[1]

H. EARL THOMAS[2] AND L. M. MASSEY[3]

ABOUT A QUARTER of a century before virus diseases as such began to be recognized, evidence of graft transmission of a chlorosis of the rose was recorded in France (*12*).[4] However, mosaic as a disease of importance in rose culture did not attract attention until about 1928 (*14*). Mosaic then for several years excited an unusual amount of comment and controversy (*7, 8, 18*) which has been only partially justified by more extensive observations and experiments (*7, 13, 17*). It is now apparent, at any rate, that the rose may be affected by virus diseases of some importance and may serve as a potential source of virus for other plants (*10*).

The material presented in this paper relates to the mosaic type of disease only. The necrotic diseases reported from the eastern United States (*2*) and abroad (*4, 5*) have not been found in California.

SYMPTOMS

As early as 1933, evidence began to appear in this work indicating that not one mosaic disease occurs among the cultivated roses but several. Since some of these were not recognized as distinct until recently, it will not be possible to treat them separately throughout this paper. For convenience these will be designated as "rose mosaic 1," "rose mosaic 2," and "rose mosaic 3," and the corresponding viruses distinguished by their respective numbers.

In roses grown out of doors, lime-induced chlorosis without malformation is rather common, notably in the Santa Clara Valley. In such cases the leaf blade becomes uniformly yellow rather than mottled. This may obscure or inhibit development of symptoms of the mosaic diseases and may in some cases be confused with them. Diagnosis in the field is also complicated very frequently by insect injury (*7*), particularly that produced by leafhoppers.

Types of variegation are encountered occasionally, which appear to be entirely genetic in origin. Scions of one such rose (fig. 3, *A*) were grafted on *Rosa odorata* and kept under observation for several years.

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[4] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

No evidence of transmission to the stock nor of change in symptoms toward those of the infectious mosaics was ever noted.

Rose Mosaic 1.—This disease on such cultivated varieties as Hollywood, Pilgrim, and Premier Supreme (fig. 1) produces small chlorotic spots somewhat angular or fringed in appearance due to the clearing of small veins and veinlets adjacent to the spot proper. The chlorotic areas

Fig. 1.—Rose mosaic 1 in leaves of the Hollywood variety.

are more numerous at or near the midvein and often appear in greater numbers near the base of the leaflet. The leaf blade around the spot is often more or less distorted. Occasionally pale bands or lines appear on leaves of affected plants, more often out of doors, but it is not known whether these are symptoms of mosaic 1. There may be no reduction in vigor or the plant may be slightly to severely dwarfed, according to the variety and, no doubt to some extent, to the growing conditions. On the four common stocks *Rosa chinensis* var. *Manetti, R. multiflora, R. odorata,* and Gloire des Rosomanes (better known in California as "Ragged Robin," which term will be used hereafter in this paper), the symptoms

are mild, seldom exceeding in severity the small chlorotic flecks shown in figure 2. Blossoms of the top varieties may be normal or nearly so in appearance or severely dwarfed and pale in color. Usually a part of the

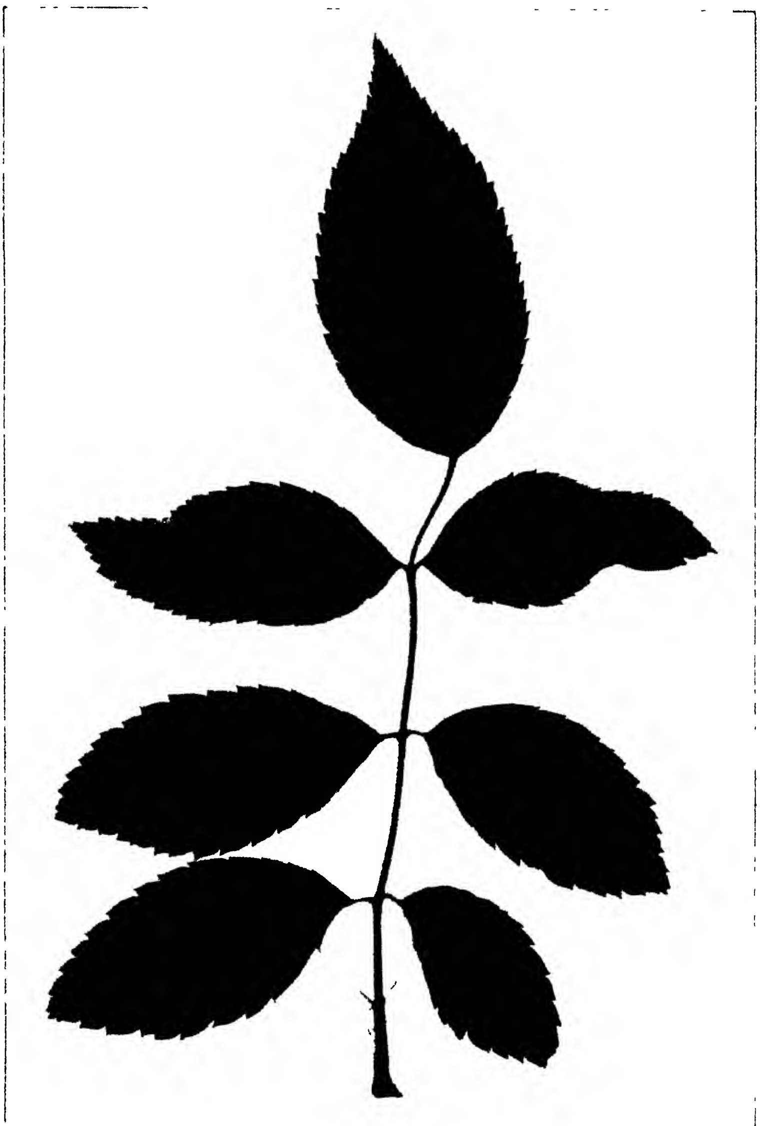

Fig. 2.—Rose mosaic 1 in *Rosa multiflora*. Only a few small chlorotic spots are present.

corolla is attenuated, leaving the flower unsymmetrical and of little commercial value. Rose mosaic 1 seems to be the principal mosaic disease of roses grown under glass, whereas mosaics 2 and 3 are more fre-

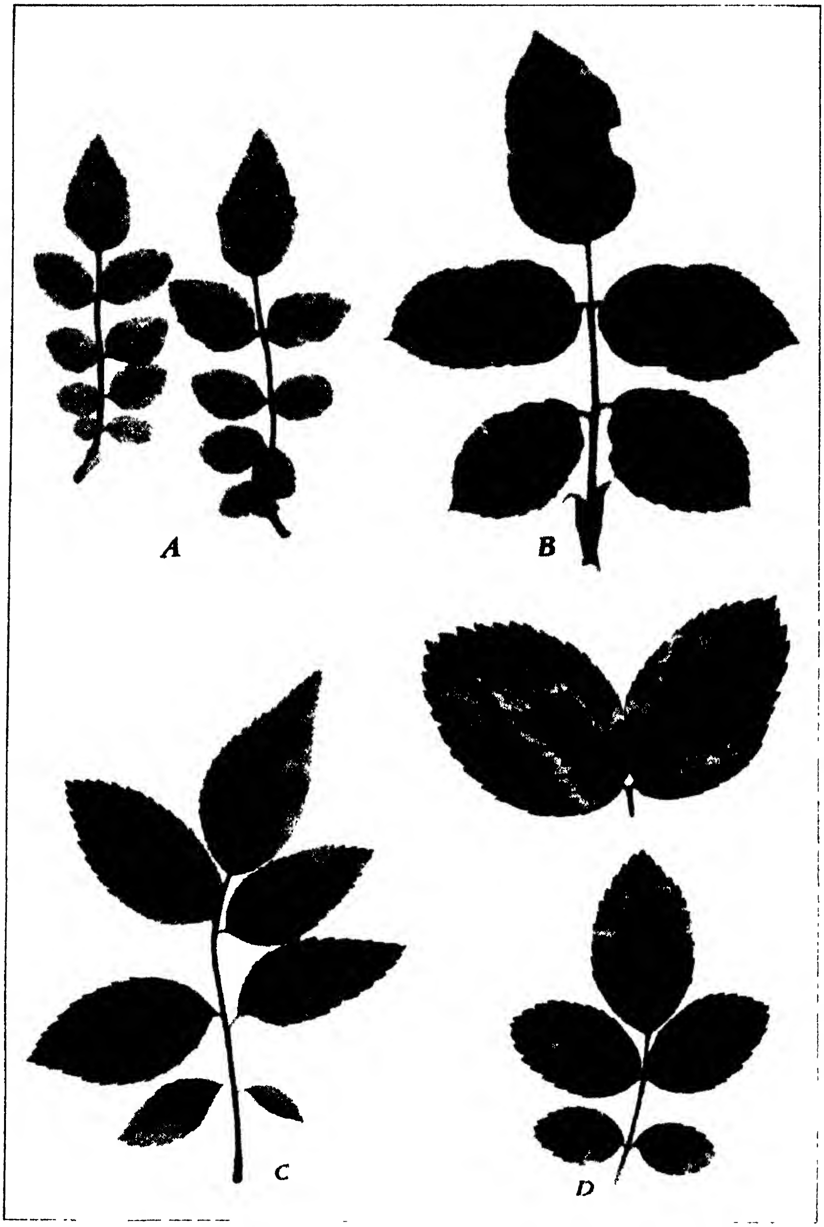

Fig. 3.—*A*, Two small rose leaves representing a noninfectious variegation; *B*, rose mosaic 2 in Belle of Portugal; *C*, rose mosaic 2 in *Rosa odorata; D*, rose mosaic 3 on *R. chinensis* var. *Manetti*. Note the oak-leaf pattern on leaves in *B* and *D*.

quently noticed in plants grown in parks and gardens. Mosaic 1 has been seen in gardens and nurseries, however, and may be more generally prevalent out of doors than is indicated by the observations to date. Only a very detailed survey early in the season could determine this point with certainty.

Rose Mosaic 2.—The disease designated as "rose mosaic 2" was first observed in the variety Cecile Brunner in a city park at San Jose. Al-

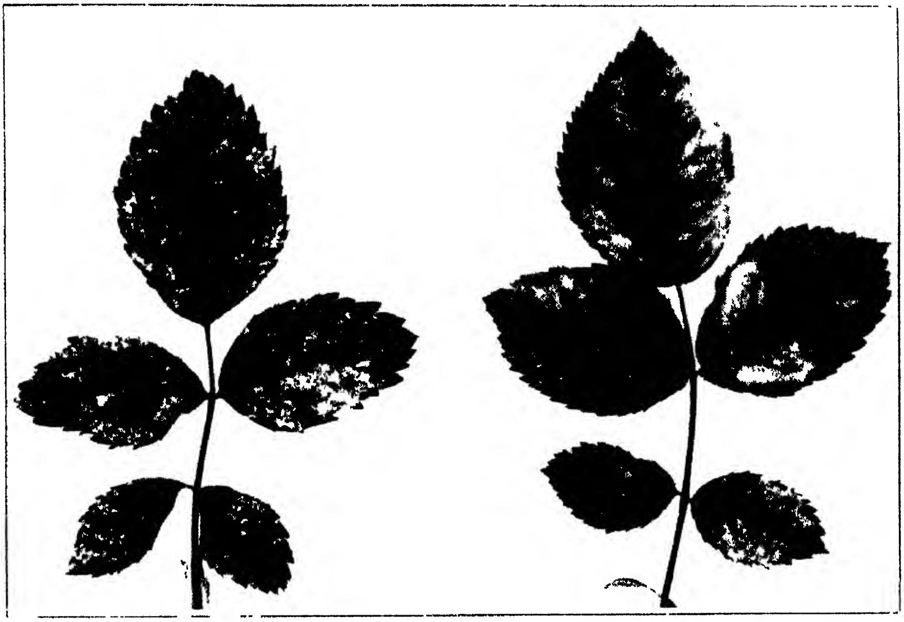

Fig. 4.—Rose mosaic 3 in Ragged Robin.

though somewhat variable in symptom expression even on the same plant, it is typically characterized in such varieties as Belle of Portugal, Cecile Brunner, Hollywood, and Independence Day by chlorotic lines, bands, and broad blotches in the leaf blade with or without distortion (figs. 3, *B, C,* and 5, *A*). The symptoms, on the whole, are distinctly more conspicuous than those of mosaic 1 on both top varieties and stocks. In some cases the disease seems to dwarf the plants somewhat; in others little or no dwarfing is apparent. No specific symptoms of blossoms have been noted for this disease.

Rose Mosaic 3.—Specimens of diseased plants designated as "rose mosaic 3" were obtained in the variety Souvenir de Claudius Pernet from a garden in Sacramento through the courtesy of D. G. Milbrath. Mosaic 3 produces symptoms on the four common stocks which are similar to those of mosaic 2 but on the whole are more severe, in some cases

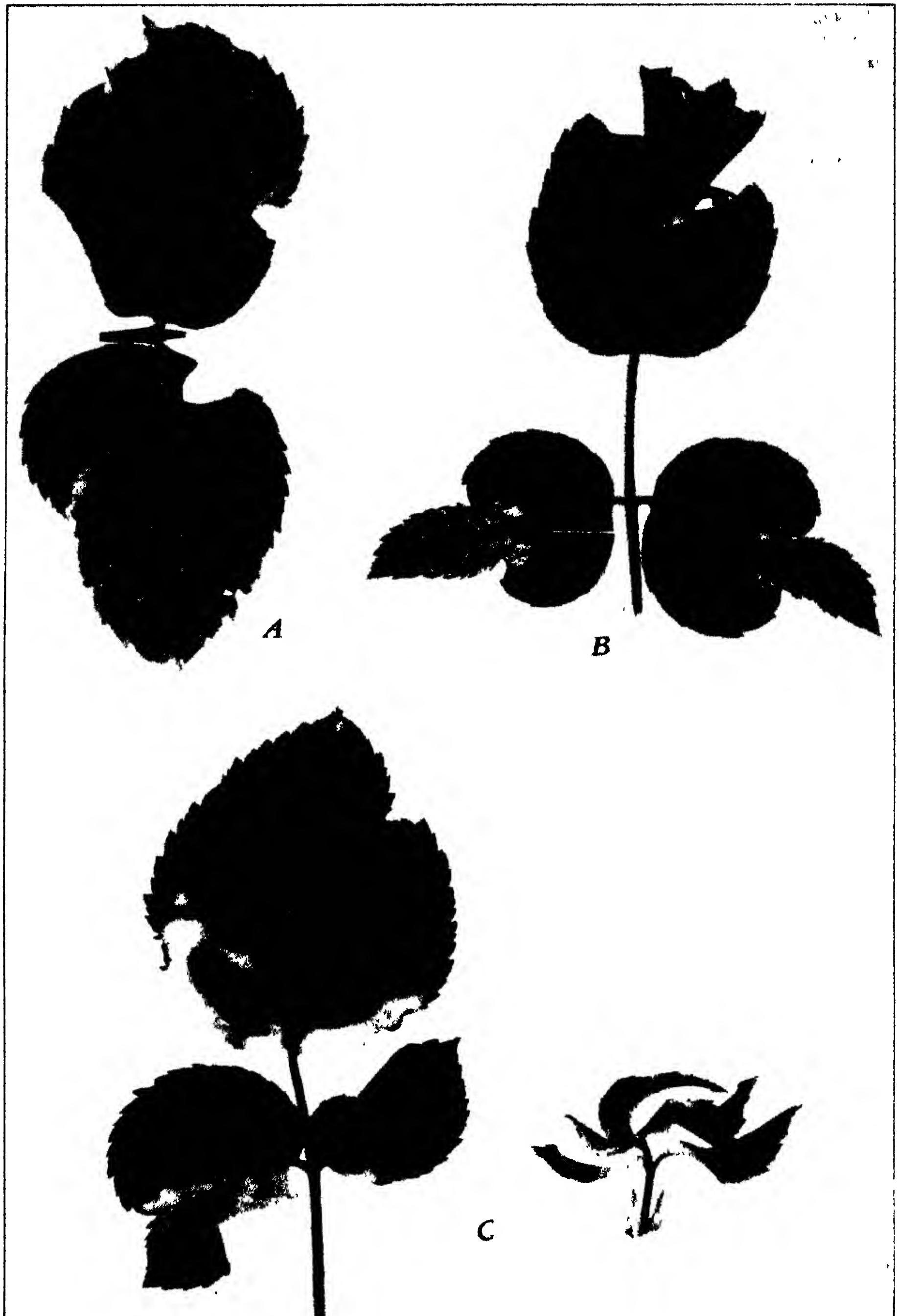

Fig. 5.—*A*, Rose mosaic 2 in Hollywood variety (pair of leaflets); *B*, apple mosaic in same variety; *C*, rose mosaic 3 in same variety, showing mottling and severe distortion.

causing distinct dwarfing. There is also more tendency toward broad chlorotic blotches in the leaf blade and few lines and rings (figs. 3, *D*, 4, and 5, *C*). Occasionally a conspicuous oak-leaf pattern (fig. 3, *D*) is produced, and not infrequently part or all of a leaf may exhibit a pronounced clearing of the veins while other leaves on the same plant bear the more common symptoms.

Other Mosaic Diseases Transmissible to Roses.—In addition to the three rose mosaics described above, the common apple mosaic is transmissible to rose (*10*) producing symptoms on Belle of Portugal similar to those of mosaic 2 but with the tendency, much more pronounced in Hollywood and Independence Day, toward marked constriction and chlorosis in a fairly definite broad band across and near the middle of the leaflet blade (fig. 5, *B*). Flowers of the Hollywood variety affected by the apple mosaic are reduced in size, and the color is lighter than normal.

Also may be mentioned here the symptoms on the rose of a peach mosaic collected at Winters, California. This mosaic is similar to but distinct from the mosaic of peach reported from Texas, Southern California, and elsewhere (*11*). The Winters peach mosaic inoculated to rose by inarching has produced marked chlorosis in leaves of Ragged Robin, sometimes rather general (fig. 6, *A*), but often limited to shorter or longer cleared areas along the larger veins. On *Rosa odorata* a few pale-green lines and rings were produced a few weeks after inoculation but these faded and did not reappear on new leaves up to more than a year from the time of inoculation. Symptoms of this disease on the Hollywood variety are similar to those on Ragged Robin, but less severe.

Several cases have come to notice which suggest the presence of still other diseases, but the relation of these to the diseases designated above has not been sufficiently tested to permit any conclusions. Among these is a specimen of Independence Day growing in a garden in Oakland, with vein clearing as the only symptom. This was grafted on *Rosa chinensis* var. *Manetti* and kept at Berkeley for more than a year alternately in the greenhouse and out of doors but remained free, or virtually so, of any other symptom and produced no symptoms on the *R. chinensis* var. *Manetti* stock.

PLANTS AFFECTED

The symptoms of rose mosaic 1 have been seen by us or illustrated by earlier workers (*7, 16*) on the following rose varieties: American Beauty, Angele Pernet, Autumn, Better Times, Briarcliff, Feu Joseph Looymans, Gruss an Coburg, Hollywood, Hortulanus Budde, Madame Butterfly, Matchless, Mrs. F. R. Pierson, Pilgrim, Premier, Premier Supreme, Radiance, Rapture, Red Radiance, Rose Hill, Southport, and

Ulrich Brunner. The symptoms on Ulrich Brunner were seen out of doors and consisted of numerous small chlorotic spots with little tendency to be aggregated near the midvein or to produce distortion of the leaflet. These symptoms persisted on this variety in the greenhouse, but when such material was used to inoculate Hollywood, the latter developed symptoms which seemed typical of mosaic 1. A considerable number of other varieties have been listed by earlier workers as subject to rose mosaic and some of them are no doubt affected by the disease here delimited as rose mosaic 1.

No attempt is made at present to classify the above varieties or those to follow according to the severity of the disease. It is apparent, however (*17*), that differences in susceptibility do exist among varieties. For example, the variety Mrs. Charles E. Russell was inoculated with each of the viruses 1, 2, and 3. Only mild symptoms, at most, were produced by any one of these. Mosaic 1 in Independence Day produced no symptoms at all, although the virus was shown to be present.

Among the stocks, Ragged Robin seems to be more affected than *Rosa chinensis* var. *Manetti*, with *R. Multiflora* and *R. odorata* intermediate between them.

Potted plants of the native species *Rosa californica*, *R. gymnocarpa* and *R. nutkana* were inoculated in the greenhouse by inarching on affected plants of cultivated varieties. No symptoms have been seen on the inoculated plants up to 12 months from the time of inoculation. Attempts to recover the virus from these have not been completed. A similar result was obtained with seedling of *R. Soulieana*.

Because of the similarity of symptoms of the other two mosaics, the probability of considerable variation in symptoms of each of them in different varieties, and the fact that most of the field observations were made before the distinction between rose mosaics 2 and 3 became apparent, all of the rose varieties which were noted as exhibiting chlorotic lines, bands, and broad blotches in the leaf blade are here grouped together. They are: Belle of Portugal, Briarcliff, Cecile Brunner, Dazla, Dorothy Perkins, Duchess of Wellington, Duchess of York, Etoile de Hollande, General MacArthur, Golden Dawn, Golden Ophelia, F. J. Grootendorst, Hadley, Hollywood, Independence Day, Irish Elegance, Irish Fireflame, Kaiserin Auguste Viktoria, Lady Margaret Stewart, Los Angeles, Louise Catherine Breslau, Mme. Edouard Herriot, Mme. la Générale Ardouin, Mrs. Aaron Ward, Mrs. E. P. Thom, Mrs. Henry Bowles, J. Otto Thilow, Paul's Scarlet Climber, Pink Cherokee Rose, Queen Alexandra, Souvenir de Claudius Pernet, Sparkler, Sunkist, Talisman, Ville de Paris, and William F. Dreer. It is entirely possible

that some of the varieties listed here were affected by diseases other than mosaics 2 and 3 and not yet differentiated from them.

The four common stocks are readily infected by rose-mosaic viruses 2 and 3. The symptoms of both diseases are somewhat less conspicuous on *Rosa chinensis* var. *Manetti* than on *R. odorata* and Ragged Robin. One strain of *R. multiflora* produced mild symptoms with mosaic 3 and somewhat stronger mottling with mosaic 2. A variety received under the name *R. multiflora Grifferaie* developed strong symptoms with mosaic 3.

Plants of the native species *Rosa californica* and *R. nutkana* inoculated with rose mosaics 2 and 3 by inarching have not shown any symptoms up to 18 months after inoculation.

The apple mosaic has been seen by us only on the rose varieties Belle of Portugal, Hollywood, and Independence Day inoculated in the greenhouse. The susceptibility of *Cotoneaster Harroviana, Eriobotrya japonica, Photinia arbutifolia,* and *Sorbus pallescens* to apple mosaic after inoculation by grafting has been pointed out in an earlier paper (*10*). Since that time striking symptoms have been obtained by inoculation on *Pyrus spectabilis,* and mild symptoms on a *Sorbus* purchased under the name *S. sitchensis* but in appearance suggesting *S. aucuparia.*

The Winters-peach-mosaic virus appears from inoculation tests to have a rather extensive range of susceptible plants including apricot, almond, and peach, as well as the roses that have been infected by inoculation. No symptoms have been found on a number of rose varieties growing in a garden adjoining a peach orchard in which this disease has been present since 1936 or earlier. Attempts to transmit this disease from peach to *Rosa californica, R. multiflora,* and *R. nutkana* have not produced visible symptoms.

Small-scale attempts were made to transmit viruses 1, 2, and 3 to peach seedlings by inarching (8 plants all told). Symptoms on the peaches were doubtful at most, and attempts to recover the viruses have failed.

Seedling apple trees were likewise inoculated with viruses 2 and 3. No evidence of infection with rose mosaic 2 was apparent up to 14 months from the time of inoculation. Of ten plants inoculated with virus 3, only one developed marked mosaic symptoms the following spring, similar in some respects to the common apple mosaic but lacking the pronounced vein clearing of that disease and tending more toward the production of chlorotic lines and rings (fig. 6, *B*). The apple variety Golden Delicious, which is highly susceptible to the apple mosaic, did not develop any clear symptoms during 15 months after inoculation with virus 3 by inarching with the affected apple seedling.

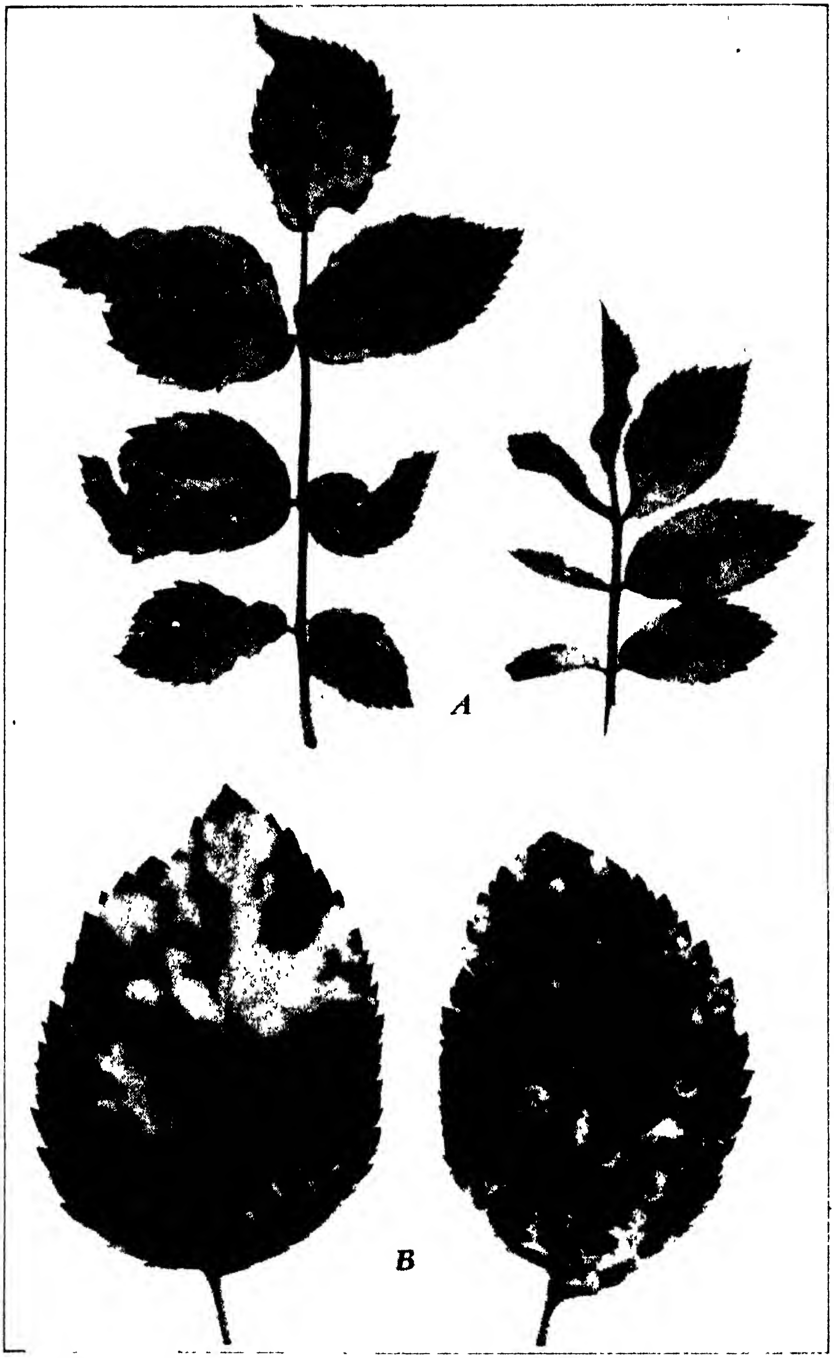

Fig. 6.—*A*, Symptoms of Winters peach mosaic in Ragged Robin rose produced by inoculation in greenhouse; *B*, rose mosaic 3 in seedling apple leaves.

BASIS FOR THE SEPARATION OF DISTINCT MOSAIC DISEASES IN THE ROSE

The tendency has been in the past to group all the mosaic symptoms of the rose together as representing a single disease, although several workers have apparently suspected the existence of more than one disease (*3, 16*). It is desirable, therefore, to present more specific evidence in support of the above designation of separate diseases.

Rose mosaic 1 is readily separable from the other diseases considered here by symptoms alone, as well as by the results of inoculations of key varieties and species. Evidence of the latter kind was obtained by grafting scions affected by this disease on healthy plants of Cecile Brunner and Independence Day. Such combinations have been grown for as long as four years without the development of any definite symptoms on these two varieties. On the other hand the same varieties develop strong symptoms when inoculated with virus 2.

The separation of mosaics 2 and 3 is more difficult. Both these diseases and the mosaics of apple and peach may at times produce symptoms on roses which are very similar. On the variety Hollywood, rose mosaics 2 and 3 may be distinguished fairly readily by symptoms alone when compared side by side under similar environment (figs. 5 *A*, *C*). The reaction of Belle of Portugal is also of assistance. With the onset of disease in this variety, rose mosaic 3 produces short necrotic lines or bands along and including the mid-vein and larger lateral veins of younger leaves and later considerable distortion of the leaf blade. The disease then becomes systemic and produces largely chlorotic symptoms. This reaction has not been noted with mosaic 2 nor with the apple mosaic in this variety. Also the Souvenir de Claudius Pernet variety, which is one of the most susceptible to mosaic 3 of those tested, has failed to show any symptoms of mosaic 2 up to 18 months from the time of inoculation.

The apple mosaic is separable from the others by the extremely slow rate of development in the rose, requiring 20 to 27 months to produce symptoms 6 inches below the point of inoculation. The symptoms of this disease are also distinctive in certain respects, notably in the varieties Hollywood and Independence Day (fig. 5, *B*), in which the chlorosis and constriction in a localized area across the leaflet is frequently seen.

The Winters peach mosaic may be separated from rose mosaics 2 and 3 by the apparent failure of viruses 2 and 3 to infect peach, the absence of lines and rings when the peach virus is in Hollywood and Ragged Robin, the presence of discontinuous chlorotic bands along the veins, and the marked tendency of chlorotic areas to become green with age.

RELATION OF STOCKS TO DISEASE IN SCION VARIETY

In a preliminary trial, scions of Pilgrim and Premier Supreme affected by rose mosaic 1 were grafted on the stocks of *Rosa chinensis* var. *Manetti, R. multiflora, R. odorata,* and Ragged Robin. Although some of these plants were kept under observation for several years, there was no indication that the stock influenced the severity of the disease in the scion variety.

That such a case may be found, however, is indicated by the fact that Belle of Portugal, affected by mosaic 2 and grown on its own roots, has shown less severe symptoms than the same variety with the same disease grown on *Rosa chinensis* var. *Manetti* and Ragged Robin rootstocks.

SYMPTOMLESS CARRIERS

As is the case with many mosaic diseases, all of those under consideration here may fail to exhibit symptoms in some or all of the leaves of an affected plant at any given time. With all except rose mosaic 1, symptoms seem to be favored by relatively low temperatures and tend to be masked at higher temperatures. No controlled experiments have been made on this point, however. Mosaic 1 is often masked for considerable periods in the common rootstocks. A few instances have been noted in which top varieties (Cecile Brunner, Independence Day, Mrs. Charles E. Russell, Souvenir de Claudius Pernet) have been exposed by grafting to virus 1 and kept so for many months (as much as four years) without exhibiting symptoms at any time. In one such case, the virus was recovered from a plant of Independence Day by grafting a healthy Hollywood scion on the side opposite the point of inoculation. The Hollywood scion promptly developed symptoms showing that the virus is at least able to pass through the Independence Day.

DISSEMINATION

Beyond budding and grafting, the method of spread of the mosaic viruses in the fields and greenhouses is not known. Particular emphasis has been placed by several workers on the shipment of rootstocks as a means of dissemination. This is no doubt of some importance, particularly with virus 1 which, at the most, produces relatively inconspicuous symptoms on the common rootstocks. Surveys in the field are not likely to be of much assistance in determining the prevalence of rose mosaic 1 in the stocks unless these are already budded to the more susceptible top varieties. In the course of this work, 3 lots of *Rosa chinensis* var. *Manetti* and 1 each of *R. odorata, R. multiflora, R. Multiflora Grifferaie,* and

Ragged Robin have been used in various experiments, including grafting to healthy top varieties, without any evidence that any of them had previously been infected by mosaic 1.

Some observations indicate, on the other hand, that the budwood of the top variety has not received sufficient attention as a source of virus (*13, 15*). For example, in a nursery where the plants were budded in place in the nursery row, rose mosaic (2 or 3) occurred in groups of 3 to 5 consecutive plants in the row, each group representing about the number that would result from a single bud stick. Also may be cited the case of a grower of roses in greenhouses who made a particular effort about five years ago to secure mosaic-free rootstocks. This was apparently done, since these stocks have been grown at Berkeley for several years both with and without grafting to healthy top varieties and have never produced any mosaic symptoms. Nevertheless, mosaic 1 continues to be more or less prevalent in some of the varieties raised by this grower.

One grower pointed out a fact which has probably led to the selection of diseased plants, in some cases, as sources of budwood. An experienced rose grower is able to detect at an early stage the defective buds that appear on affected plants (mosaic 1). Whether or not he is aware of mosaic, these buds are removed in the hope that the plant will produce other normal buds before the cutting season is past. In a variety that is not greatly reduced in vigor by the disease, this practice leaves the affected plants at the end of the harvest season larger and more vigorous in gross appearance than adjacent healthy plants which have been heavily cut for the flowers, and leads in some cases to the singling out of these diseased plants as a source of buds for propagation.

HEAT TREATMENTS OF AFFECTED CUTTINGS

Although exposure to high temperatures has been used successfully in only a few cases (*6*) in inactivating virus in vegetative plant parts, this remains the only method of any particular promise. One test with negative results has been reported for rose mosaic (*9*). The results obtained at the California Agricultural Experiment Station with roses are negative thus far and will be treated as briefly as is feasible.

Virus 1 survived the following three treatments when the cuttings did; but many of the cuttings died.

a) Cuttings were planted in a cutting box in sand held at approximately 30° C. The cuttings were completely covered by the sand for initial periods of 11 and 26 days and then uncovered at the tip, followed in the latter case by an additional period of 53 days in the warm sand which dropped to about 28° C toward the end of the period.

b) Cuttings in moist sphagnum and wrapped in waxed paper were held for 9 and 14 days at 36° C.

c) Cuttings were immersed in water at 45° C for 15 and 30 minutes.

Cuttings exposed to an air temperature of 55° C for 30 and 60 minutes did not survive. The cut surfaces were covered by an asphalt emulsion during the treatment.

Virus 2 survived in cuttings completely covered for 11 days with moist sand at 30° C. Cuttings with the basal ends in water exposed for 15 and 30 minutes to an air temperature of 55° did not survive.

Cuttings affected by mosaic 3 immersed in water at 45° C for 45 and 90 minutes remained alive for as much as 4 weeks but all died without making any growth.

DISCUSSION

Since rose mosaic 1 seems to be the common disease of greenhouse roses capable of causing direct loss in yield of desirable flowers, and since the other diseases under discussion are sufficiently conspicuous to be more easily avoided in the selection of cuttings and budwood of both stocks and top varieties, the former is in particular need of further study. The stocks grown out of doors are soon marked, more or less, by the feeding of leafhoppers and other insects. This obscures largely or entirely the symptoms which are, at best, discernible with difficulty by any means except grafting with a known susceptible variety. More specifically, the determination of the identity and habits of the vector of mosaic 1 seems imperative for any program looking toward the maintenance of disease-free stocks.

In view of the number of distinct diseases which have emerged in the course of this work from a small number of collections, it seems probable that much is yet to be done in the separation of specific mosaic diseases of the rose and in the determination of their relation to diseases of other plants.

The adoption of the somewhat paradoxical procedure of selecting a more susceptible stock may prove advisable in order to facilitate the eradication of rose mosaic 1 by roguing. The resistance of certain species and varieties to particular diseases, however, suggests the possibility that rose stocks may eventually be found which are not even symptomless carriers of these diseases.

For the immediate future, a more careful selection of budwood seems to be the obvious way of greatly reducing mosaic 1 in roses to be grown in greenhouses. Since this disease seems to spread relatively slowly in greenhouses where insect control is consistently practiced, the roguing out of diseased plants during the first season in the greenhouse is indi-

cated. In a greenhouse where 50 per cent of the plants were infected originally, one worker has reported (*1*) the reduction of the disease to a minimum by roguing. Plants removed at an early stage can be replaced, or failing this the neighboring densely set plants will often occupy most of the available space or all of it.

SUMMARY

Three distinct mosaic diseases of the rose were found in central California. These are designated as rose mosaics 1, 2, and 3. Methods for distinguishing the diseases from each other are presented. In addition, roses were infected by inoculation with apple-mosaic virus and the virus of a disease of peach designated as "Winters peach mosaic."

The use of buds from diseased plants seems to be an important means of introducing the diseases.

The virus of rose mosaic 1 survived heat treatments which were near the limit of tolerance of the rose cuttings. Virus 2 withstood exposure at 30° C for 11 days.

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H I L G A R D I A

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THE RÔLE OF SURFACE TENSION AND CONTACT ANGLE IN THE PERFORMANCE OF SPRAY LIQUIDS[1,2]

WALTER EBELING[3]

INTRODUCTION

The wetting and spreading properties of spray liquids have been defined by Martin (*16*)[4] as follows:

(*a*) *Wetting properties* are defined by the ability of the liquid to form a persistent liquid-solid interface when excess of liquid is drained from the surface and are assessed by the receding contact angle. Perfect wetting results when this angle is zero. ($\cos \theta_r = 1.$)

(*b*) *Spreading properties* are defined by the ability of the liquid to form a persistent liquid-solid interface solely by surface activity over the plain solid surface, and are a function of the advancing contact angle.

Both wetting and spreading are functions of the contact angle, in one case the receding[5] angle, and in the other case the advancing angle.

In the course of experimental work on the insecticidal efficiency of various aqueous solutions the writer has become interested in the spreading and penetrating properties of the liquids as indicated by their advancing contact angle.

An attempt has been made to devise a means of contact-angle measurement which would combine practicability with the great accuracy

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[4] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

[5] When a drop of liquid is placed on a solid, it may either spread over the surface of the solid in a thin film or it may come to rest before spreading out into a film, in which case there is a contact angle between the surface of the liquid and the solid. This angle is called the *advancing* contact angle. If sufficient liquid is withdrawn from the drop flattening it further, or if the drop is inclined the *receding* contact angle is formed.

necessary for measurements of the low contact angles common to all spray oils as well as to aqueous solutions with low surface tension. The present paper includes an explanation of this method and a presentation of results of experiments relating to differences in the contact angles of various liquids; the influence of various types of substrata and the effect of the addition of solutes are also considered.

The writer is aware of the fact that the static contact angle may not be an accurate criterion of the wetting and spreading ability of a liquid under the dynamic conditions existing when a spray is being applied. Ben-Amotz and Hoskins (*2*), by comparing the static and dynamic behavior of oil emulsions on beeswax surfaces, have shown that although oil emulsions containing 0.0225 per cent sodium oleate have a much lower static contact angle than those with equivalent concentrations of blood albumin and hemoglobin, they do not wet as well when applied as a spray.

Nevertheless, after a spray liquid is applied to a surface, its further spread and its penetration into masses of waxy threads, such as cover mealybugs and aphid colonies, or its penetration into the spiracles of insects, under the bodies of scale insects, and into plant tissues is influenced by its advancing contact angle under more or less static conditions. This follows from the laws of capillary flow, and can be proved experimentally, as will be shown later. Consequently, in connection with many of the entomological problems involving the use of sprays, contact angle is a good index of the physical qualifications of the spray liquid.

PREVIOUS METHODS OF CONTACT-ANGLE MEASUREMENT

Stellwaag (*20*) utilized a principle, worked out by H. P. Wilson (see Sulman, *21*), in determination of the contact angles of various spray liquids on leaf surfaces and insect integuments. The liquid to be tested was placed in a container, and the solid (leaf surface, insect integument, etc.) was attached to a device for holding it and dipping it into the liquid until the surface of the liquid was exactly horizontal at the point of contact with the solid. The image of the object was reflected on a mirror upon which was etched a protractor; from this the contact angle could be read. This method was adopted, with refinements, by English (*9*).

O'Kane *et al.* (*17*) devised a method of photographing a drop of spray liquid in contact with the integument of an insect or with any other solid. They were able to make a photograph six or eight seconds after a

drop had been placed on a solid and to make subsequent photographs of the same drop at intervals of a few seconds. When the contact angle was measured, the negatives of the photographs, which were 24×36 mm in size, were projected on a screen, the image thus being enlarged about 150 times the diameter of the original drop. The contact angle was then determined by drawing a tangent to the point of contact, liquid/solid, and by measuring with a protractor the angle made with the solid by the tangent. Bartell and Merrill (*1*) have employed a similar method in measuring the contact angles of liquids in capillary tubes.

The writer believes that the microprojection of liquid drops in the manner described in the present paper makes possible a simpler and more rapid method for the determination of static contact angle than the methods which have been employed to date.

MICROPROJECTION OF DROP IMAGES

The Principle of the Microprojector.—The apparatus to be described herewith is designated as the "Triple-Purpose Microprojector" (fig. 1), which was designed primarily for the projection of mounted specimens on slides. Images may be projected to a distance of from 4 to 15 feet; the range of magnifications is then from 30-fold to 230-fold. For drawing, however, a mirror reflects the image onto a notebook or sheet of paper directly below it.

A lamp house containing a special 6-volt bulb is adjustably attached to the optical bed of the projector, and the condensing system is attached to the front of the lamp house. For the finer focusing, the objectives are held in a focusing mount operated by a handle traveling in a helical slot; for coarse adjustment, the entire objective holder is moved along the optical bed. This type of adjustment is needed for the accommodation of objects other than microscope slides, the latter being inserted in a fixed position on an object stage rigidly attached to the optical bed in front of the lamp house.

Adaptation of Microprojector for the Projection of Drop Images.—In figure 1 is shown the setup employed for the projection of liquid drops. A cork upon which is glued a platform for glass slides is held in place by a clamp adjustably connected with the vertical rod of a ringstand and placed in front of the object stage, while the objective holder is moved along the optical bed to obtain the proper focus of the image. The object on which the drop is to be placed is put on the platform, which is very easily adjusted into a horizontal position with the aid of a level. In the present investigation, microscope slides were cut into halves lengthwise and, before being placed on the platform, the portion of the

slide to be used was coated with the desired substratum for the drop, such as beeswax, paraffin wax, leaf wax, or scale-insect wax. Also a smooth piece of glabrous leaf, such as the portion between two parallel veins, was sometimes placed on the slide and held in place by thin pieces of glass which were in turn held in place by rubber bands.

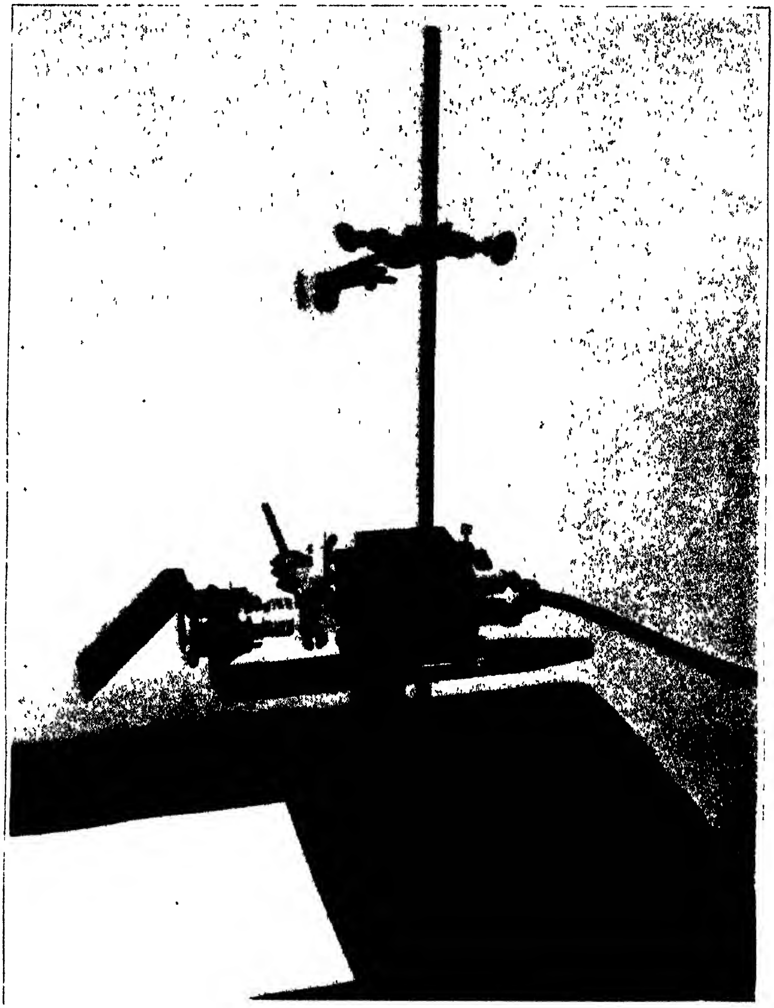

Fig. 1.—Microprojector used to project the images of liquid drops in contact-angle determinations. Description in the text.

In the present investigation, the microprojector was elevated in position by placing it on a box so that the sheet upon which the image was reflected was 34 inches below the mirror; at this distance the image was magnified about 50-fold.

Liquids of high contact angle may be withdrawn from a flask by means of a small pipette, or by means of a splinter of wood tapering to a point about a half millimeter in thickness at one end. Drops with lower contact angle such as oil, or water with reduced surface tension, may be transferred by means of a ruling pen. By touching the substratum with the pen a drop of liquid can be deposited. This can gradually be increased to the desired size by repeatedly touching the same drop with the point of the pen. The volume of liquid given off by the pen can be controlled by a screw regulating the proximity of the spring blades of the pen.

The image may then be traced when the drop has come to rest. The drop outline may be traced or points may be accurately placed on the sharply defined curve outline. The contact angle of the drop will be at its maximum value, for the advancing contact angle is being measured. Evans and Martin (*10*) have shown that it is of little importance whether the advancing or receding equilibrium contact angles are used in comparing wetting agents.

It is convenient to trace the drop outline on cross-section paper, for one of the horizontal lines may then serve as a base for the curve of the drop outline. If the contact angle is to be derived mathematically, it is convenient to place the cross-section or coördinate paper so that one extremity of the curve will rest at the origin of the coördinate axes and the other extremity will rest on the X axis; thus, the coördinate values of different points may be read on the same paper on which the curve is traced.

If the droplet is placed on the substratum on the platform held by the clamp as in figure 1, the temperature in the immediate environment of the drop is about 80° F. Careful observation of the drop image shows that there is no appreciable evaporation of aqueous solution during the time necessary to trace the image. Evaporation of spray oil is, of course, even less rapid. The fact that the temperature is fairly constant, despite minor variations in room temperature, is an advantage in experimental work. Considerable variation in temperature, however, has a very slight effect on contact angle. Du Noüy (*5*, p. 29) states that at around 20° C. each degree of temperature added or substracted corresponds to a difference of only 0.17 dyne in surface tension of an aqueous solution. With more viscous liquids, however, a more remote source of light might be an improvement over the present apparatus in order that heating of the solution above room temperature might be avoided.

MEASUREMENT OF CONTACT ANGLE

Empirical Methods.—Such good definition of the outline of the drop of liquid projected by the microprojector can be obtained that the curve traced on the cross-section paper on which the image of the drop is projected is a very accurate representation of the actual curvature of the drop on the particular substratum in question. Any error in the measurement of the contact angle, therefore, must be that which is inherent in human manipulation. Differences as great as 5½ degrees were noted in measurements made by means of a protractor by various persons independently measuring the contact angle of a single drop outline.

A greater magnification, as obtained by O'Kane *et al.* (*17*) by projecting negatives of the drop images on a screen with a consequent magnification of the original drop of 150-fold, reduces the error of measurement but does not eliminate it, besides having the disadvantage of requiring special apparatus and delay in obtaining the required values.

It appeared to the writer that accurate values could be obtained not from refinements in measuring technique, but from mathematical derivation.

If distilled water is dropped on the surface of a cabbage leaf, the free surface of the water will describe a portion of an ellipse, the shape of which is affected by the side of the leaf upon which the drop happens to be placed (fig. 2, *A* and *B*).

The influence of gravity diminishes as the drop becomes smaller. Even for comparatively large drops (about 5 mm in diameter) the deviation from the spherical shape caused by gravitation becomes less as the contact angle is reduced. As the height of the vertex of the drop becomes less, gravitational effect on configuration of the drop is correspondingly reduced, until when the drop has a contact angle of about 65° (fig. 2, *C*) the effect need not be considered for practical purposes, as will later be shown mathematically.

The drop curvatures dealt with in connection with the present investigation were in every case shown to be exact expressions of ellipse equations. This, in turn, suggested an exact method for the derivation of the contact angle. Since the general equation for the ellipse when its axis is parallel to one of the coördinate axes is $Ax^2 + Bx + Cy^2 + Dy + K = O$, the equation for any given drop curvature may be calculated from the coördinate values of only four points of a curve passing through the origin, from which four simultaneous equations may be derived and four unknowns solved for in terms of the fifth, which may be cancelled out and the resulting figures substituted in the general equation. As

stated before, the coördinate values may be taken directly from the original tracing of the drop outline if it is traced on coördinate paper.

The general equation for the circle is $x^2 + Bx + y^2 + Dy + K = O$. If the curve sufficiently approximates a circle, the coördinate values of only three points are necessary, since only three unknowns need be

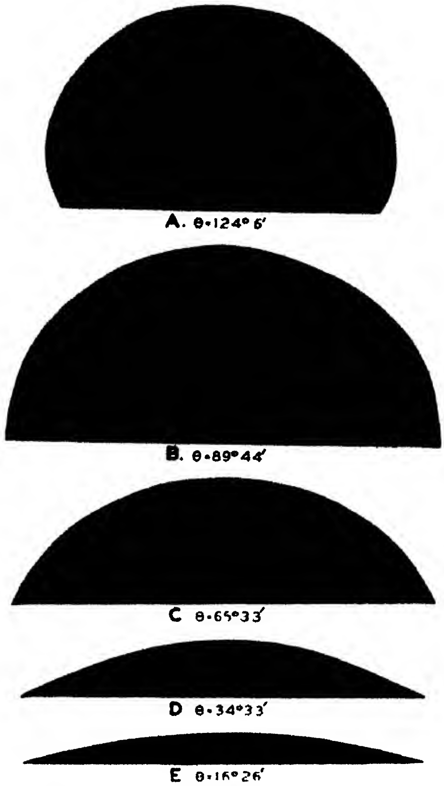

Fig. 2.—Projected outlines of liquid drops. *A*, Water on the ventral surface of a cabbage leaf; *B*, water on the dorsal surface of the same leaf; *C*, water on the dorsal surface of a lemon leaf; *D*, grade 5 (heavy) spray oil on the ventral surface of an orange leaf; *E*, kerosene on the ventral surface of an orange leaf.

solved for. Also, since x^2 and y^2 have no unknown coefficients, the simultaneous equations may be equated to numerical values and the unknowns may be solved for directly.

When the equation of the curve has been computed, differentiation of the equation at $x = O$ will give the slope of the curve at its origin, which is the point of contact, liquid/solid. This value is, of course, the

tangent of the angle of contact, which may then be found by referring to a table of the natural trigonometric functions.

Mack's Formula.—Mack (*15*) has shown that for a drop whose outline is a segment of a sphere, the contact angle may be calculated from the equation $\theta = 2 \tan^{-1}\left(\frac{h}{x}\right)$ where h is the greatest height of the drop and x is the radius of the base of the segment. He states that if it is 0.5 mm or less in diameter, a sessile drop may be considered a segment of a sphere, except for contact angles near 180°. However, it is apparent from inspection of the equations of drop outlines which have been determined by differentiation in the present investigation[6] that for drops as large as 5 mm in diameter, all those having a contact angle less than 65° are for practical purposes segments of spheres. The contact angles of these drops may therefore be calculated by Mack's formula with a great saving of time as compared to the differentiation of the circle equation. Mack's formula was employed in the computation of all the contact angles which are to follow in this paper. By making the drops sufficiently small say 2 or 3 mm in basal area, even drops with contact angles of around 90° are sufficiently spherical for practical purposes so that Mack's formula may be used in calculating their contact angles.

If reasonably small drops are placed on a solid, a considerable variation in their volumes does not appear to affect their contact-angle values. If too large a drop is placed on a solid, the momentum given to the larger mass of liquid by gravity will cause the contact angle to be reduced to various values within the range of hysteresis. This difficulty may be overcome by waiting until gravitational momentum asserts itself, then building the contact angle up to its maximum value by adding more liquid to the original drop. The independence of contact angle and drop size is in accord with the fact, as Freundlich (*11*) has pointed out, that surface tension remains constant with increase in surface, an important contrast to the tension of a stretching membrane, to which surface tension is sometimes compared.

The writer has found no greater variation in the value θ for liquid

[6] The equations for the outlines of the drops in figure 2 are for *A*, *B*, *C*, *D*, and *E*, respectively, as follows:

$0.47x^2 - 29.56x + y^2 - 20.15y - 8.08 = 0;\ \theta = 124°\ 6'$

$0.82x^2 - 58.92x + y^2 + 0.273y - 12.31 = 0;\ \theta = 89°\ 44'$

$0.98x^2 - 63.33x + y^2 + 28.80y - 31.10 = 0;\ \theta = 65°\ 33'$

$x^2 - 64.55x + y^2 + 93.93y - 59.87 = 0;\ \theta = 34°\ 33'$

$x^2 - 62.00x + y^2 + 210.00y - 39.00 = 0;\ \theta = 16°\ 26'$

As the contact angle changed from 124° 6′ to 65° 33′ the coefficients of x^2 and y^2 became more nearly identical, showing that the ellipse gradually became a circle as the height of the drop diminished with a consequent diminution of the effect of gravity on the shape of the drop.

drops of high contact angle than for those of low contact angle. Thus the θ values for 5 drops of distilled water on beeswax-coated glass slides were found to be 88° 4′, 88° 32′, 89° 16′, 90° 0′, and 87° 34′, an average of 88° 41′ ± 21′. The drops were about 3 mm in diameter. Next 5 drops of grade 4 (medium) spray oil, also about 3 mm in diameter, were placed on a beeswax-coated glass slide and their images projected in the same way as before and under identical conditions (temperature of liquid, 72° C; temperature of air at the point where the drop was placed, 80° C). The computed values for the contact angles were 30° 28′, 31° 56′, 33° 18′, 32° 18′, and 31° 42′, an average of 31° 56′ ± 20′. Approximately the same amount of variation occurred among the 5 measurements in both tests.

A STATISTICAL TEST OF THE RELIABILITY OF THE METHOD

Beeswax surfaces were made by dipping portions of glass microscope slides, made by cutting the standard slide in two parts lengthwise, into melted beeswax at 100° C, after which the slides were placed in a vertical position to cool at room temperatures. This procedure results in a thin, uniform layer of wax on both sides of the glass slides.

TABLE 1

CONTACT ANGLES ON BEESWAX SURFACES OF FIVE DROPS OF ONE-TENTH PER CENT SODIUM OLEATE SOLUTION IN EACH OF FIVE DROP DIMENSIONS*

Width of drops, mm	Drop 1	Drop 2	Drop 3	Drop 4	Drop 5	Mean
1	27° 28′	24° 0′	24° 48′	27° 34′	24° 2′	25° 34′ ± 29′
2	25° 21′	27° 20′	25° 30′	25° 24′	27° 0′	26° 7′ ± 16′
3	26° 45′	28° 4′	27° 56′	27° 44′	27° 22′	27° 30′ ± 8′
4	25° 48′	27° 6′	27° 25′	26° 22′	26° 2′	26° 32′ ± 11′
5	24° 49′	26° 34′	24° 34′	26° 26′	27° 2′	25° 50′ ± 15′

* An analysis of variance revealed no significant differences in contact angle between the various drops of each dimension nor between the drops of different dimensions. However, a comparison of the probable errors shows that the measurements of the drops 3 mm in basal diameter were the least variable.

Drops of 0.1 per cent sodium oleate solution were placed on the beeswax surfaces immediately after the solution had been shaken in the flask. The drops ranged in diameter of basal area from 1 to 5 mm. Five measurements were made of each drop dimension and the contact angles computed from these data by means of Mack's formula. The measurements were made by a laboratory assistant[7] who had no previous experience in this type of work. The resulting figures are shown in table 1.

[7] Mr. Louis Riehl made many of the contact-angle determinations and also aided in the capillarity experiments which are to be mentioned later.

According to an analysis of variance (*19*, p. 22), no significant differences in contact angles were found either between successive measurements of a single drop dimension or between measurements of the five different drop dimensions, although the greatest variation occurred among the latter. This means that the investigator need not concern himself with the exact amount of liquid used to form the drop and that a considerable variation in drop size will not appreciably vitiate results.

TABLE 2

CONTACT ANGLES ON BEESWAX SURFACES OF THREE AQUEOUS SOLUTIONS WITH DIFFERENT WETTING AGENTS*

Material	Approximate width of drop, mm	Contact angle	Mean
Solution A	1	34° 20′	32° 18′ ± 21′
	2	32° 24′	
	3	32° 26′	
	4	31° 20′	
	5	31° 2′	
Solution B	1	41° 10′	40° 54′ ± 19′
	2	40° 48′	
	3	41° 6′	
	4	42° 22′	
	5	39° 0′	
Solution C	1	44° 32′	44° 26′ ± 25′
	2	42° 10′	
	3	46° 28′	
	4	44° 28′	
	5	46° 14′	

* An analysis of variance revealed no significant differences in contact angle between drops of different widths, but showed that the differences between the contact angles of the different solutions were highly significant.

However, a drop size of about 3 mm would probably be chosen as the most convenient size from the standpoint of ease of manipulation, and a variation of over a half millimeter in either direction would not often occur in ordinary work. The probable errors of the means (table 1) show that the least variation occurred among the measurements in the class of drop 3 mm in width and that variation increased in proportion to the deviation from this dimension in either direction. The larger drops tend to flatten out from the force of momentum to reach a contact angle somewhat below the maximum, but, as mentioned before, this difficulty can be overcome by building up the drop by adding more liquid from the ruling pen after the drop has come to rest. The 3-mm dimension, however, appears to be the most convenient and the best suited for the purpose under consideration. As can be seen from table 1, in making

five measurements of drops 3 mm in diameter, one may expect half of the contact-angle values to fall within a range of plus or minus 8 minutes. This represents a far greater accuracy than would be necessary for ordinary purposes.

Measurements were next made of the contact angles on beeswax surfaces of three aqueous solutions with 1 per cent concentrations of different wetting agents (table 2). The wetting agents were Vatsol OT[a] (solution A) and two sulfated alcohols (solutions B and C), differing only in the complexity of the alcohols from which they were derived. An analysis of variance again showed no difference in the contact angles of drops of different dimensions but showed significant differences between the contact angles of different solutions, although the difference between the contact angles of solutions A and B was only 3° 32′ ± 31′.

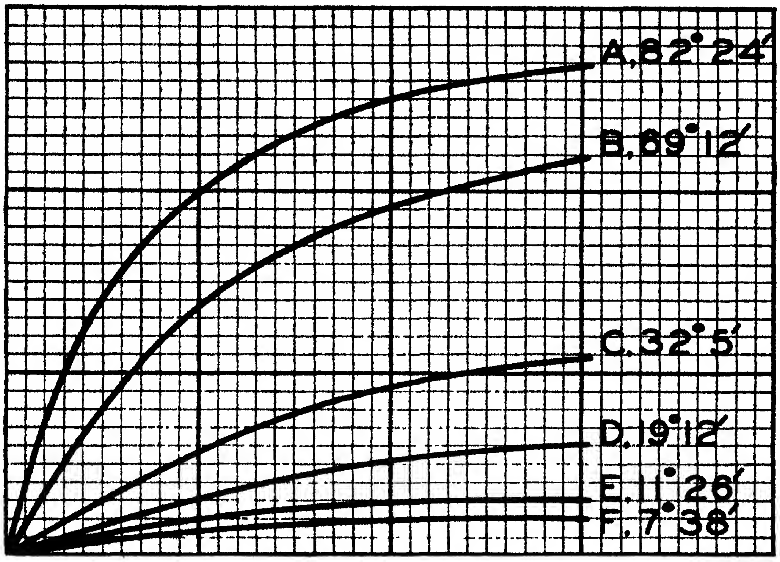

Fig. 3.—Effect of substratum on drop curvature and contact angle of liquids. Each curve shows the contour of the left half of a drop. *A*, Water on the ventral surface of a lemon leaf; *B*, water on the dorsal surface of the same leaf; *C*, grade 5 (heavy) oil on the ventral surface of a *Viburnum* leaf; *D*, grade 5 oil on the dorsal surface of a clean lemon leaf; *E*, grade 5 oil on the dorsal surface of an uncleaned lemon leaf; *F*, grade 5 oil on the dorsal surface of an apple leaf.

SOME CONTACT-ANGLE MEASUREMENTS

Effect of the Nature of the Substratum.—One of the most interesting examples of the effect of the substratum on contact angle is afforded by different leaf surfaces (fig. 3). In his exhaustive study on this subject

[a] Sodium salt of an ester of sulfosuccinic acid.

Stellwaag (*20*) showed that not only does the contact angle of a given liquid vary greatly on leaves from different plant species, but also on leaves of different ages of a single species, or on different surfaces of a single leaf. He also found that desiccation of the leaves had the effect of increasing the contact angle.

The contact angles of both oil and water are higher on young citrus leaves than on older leaves. Also, the contact angles of both oil and water

TABLE 3

VARIATIONS IN THE CONTACT ANGLES OF WATER AND SPRAY OIL ON THE DORSAL AND VENTRAL SURFACES OF THE LEAVES* OF THREE CITRUS VARIETIES

Variety	Surface of leaf	Liquid†	Contact angle‡
Orange	Dorsal	Water	60° 57′ ± 1° 28′
Orange	Ventral	Water	69° 54′ ± 58′
Grapefruit	Dorsal	Water	61° 51′ ± 1° 15′
Grapefruit	Ventral	Water	67° 21′ ± 65′
Lemon	Dorsal	Water	67° 30′ ± 2° 11′
Lemon	Ventral	Water	76° 42′ ± 2° 2′
Lemon (not cleaned)	Dorsal	Water	37° 36′ ± 4° 38′
Lemon (not cleaned)	Ventral	Water	75° 15′ ± 2° 1′
Orange	Dorsal	Oil	27° 58′ ± 59′
Orange	Ventral	Oil	31° 53′ ± 28′
Grapefruit	Dorsal	Oil	21° 20′ ± 40′
Grapefruit	Ventral	Oil	30° 11′ ± 46′
Lemon	Dorsal	Oil	25° 39′ ± 1° 16′
Lemon	Ventral	Oil	28° 32′ ± 53′

* The leaves were all from the second cycle of growth and were consequently of approximately the same age.

† Distilled water and grade 4 (medium) spray oil were used in the test The temperature of the liquids was 73° F and the temperature of the air at the point where the drop was placed for projection was 80° F. In all tests but one the leaves were washed with tap water and dried with a clean towel before the contact-angle determinations were made.

‡ The differences between the contact angles of the liquids on the dorsal and ventral sides of each leaf were significant.

are greater on the ventral sides of citrus leaves of the second growth cycle than on the dorsal (upper) sides (table 3). Thus a spray solution will roll into beads on the ventral sides of citrus leaves, while the same solution may wet the leaf in an even film on the dorsal side of the same leaf. The spray solution will also "bead" more on younger leaves than on older leaves. The probable errors in table 3 show that, with a single exception, the contact angles of both the water and oil drops are more variable on the dorsal than on the ventral sides of the leaves.

Table 3 shows that the contact angle of water on the ventral sides of citrus leaves is higher than on the dorsal sides; it does not seem, however, that the differences which were found on clean leaves are in proportion to the great differences between the spreading of spray solutions on the dorsal and ventral sides of citrus leaves which may be observed

when spraying trees. Under actual field conditions the dorsal sides of the leaves have a layer of orchard dust, while the ventral sides have practically none. Since water spreads well on a layer of soil particles, the dorsal sides of the leaves are readily wetted. The test (table 3) in which the leaves were not washed showed that on leaves as they occur in the orchard, the differences in the contact angles of water on the dorsal and ventral sides of the leaves are much greater than on clean leaves.

As stated before, a uniform substratum may be obtained by dipping a microscope slide into melted wax and keeping the slide in a vertical position while the coating of wax is cooling, or by dipping the slide into

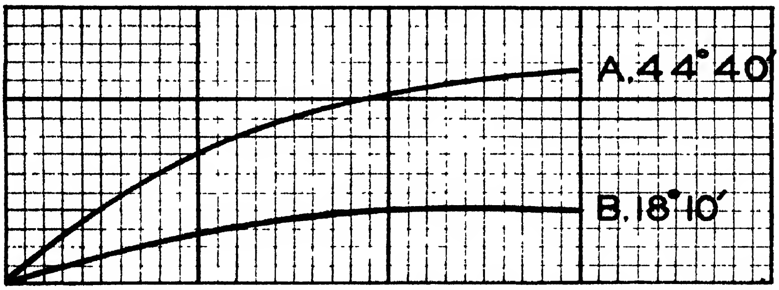

Fig. 4.—The drop curvatures and contact angles of grade 5 (heavy) spray oil on extracted leaf wax (*A*) and extracted red-scale wax (*B*).

a solution of wax and carbon tetrachloride (25 per cent wax and 75 per cent carbon tetrachloride) and placing the slide in a vertical position while the carbon tetrachloride is evaporating. In the present investigation comparisons are being made of the contact angles of various liquids on uniform surfaces of paraffin wax or beeswax. Since it was considered that a knowledge of the preferential wetting of oil with respect to citrus-leaf wax and the wax of red scale, *Aonidiella aurantii* (Mask.), would be at least of academic interest, these waxes were extracted by soaking the scales and leaves in petroleum ether for five days with occasional shaking of the solution. The scales or leaves were then removed and the ether driven off by heat.

Figure 4 shows that the contact angle of oil is much lower on scale wax than on leaf wax. However, the extract from the entire scale body may not be representative of the waxy threads exuded by the insect which comprise the mass of wax beneath the scale body through which the oil must penetrate in order to reach the spiracles.

That the oil can traverse the comparatively great distance from the margin of the scale body to the spiracles before penetrating the cuticle

of the leaf is not surprising in view of the fact that the wax on the underside of the scale body, which must be traversed by the oil on its way to the spiracles, is made up of a mass of loosely woven, waxy threads exuded by glands on the pygidium of the insect (fig. 5). In this way a great surface area per unit distance is presented to the oil, with a consequent increase in the capillary attraction. If a red scale is fixed securely on its dorsal surface, as by placing it on a thin layer of balsam, and a

Fig. 5.—Red scales removed from a lemon leaf, each placed on its dorsal side to show the ventral mass of waxy threads between the scale body and its substratum. The scale on the right was sprayed with oil, which penetrated through the mass of wax into the spiracles of the insect and consequently caused the wax to become translucent, revealing the dark body of the scale. Greatly magnified.

small drop of oil is placed on the center of its ventral surface, the oil will spread rapidly to the edges of the scale, while a drop of similar size placed on the dorsal side of a scale will spread much more slowly.

The capillary nature of the mass of wax beneath the body of the red scale (fig. 5) favors the rapid penetration of oil to the spiracles of the insect. If sufficient oil is deposited by the spray, the oil reaches the spiracles in an instant, which proves that the movement of oil to the spiracles is by capillary flow, for if the movement of oil were by penetration of solid wax, several days, at least, would be required for penetration to the spiracles. In fact, the oil would be absorbed by the substratum upon which the scale happened to be situated before it could reach the relatively long distance from the edge of the scale armor to the spiracles, which are situated at points about two-thirds the distance from the edge of the scale armor to the center of the insect.

If the scale is situated on a highly absorptive substance, such as the rough bark of citrus trees, the oil may penetrate this substance before it reaches the spiracles of the scale, despite the fact that it penetrates under the scale so readily. Consequently, greater concentrations of oil in the spray are necessary to insure the penetration of the oil to the insect spiracles.

A knowledge of the contact angle of oil on water was considered desirable in view of the fact that it is commonly conceived that each drop of water as it strikes the tree surface in the spraying operation bears on its surface numerous small lenses of oil (*18*, p. 60). This, of course, is not true of the emulsive oils, which spread in a thin film over water.

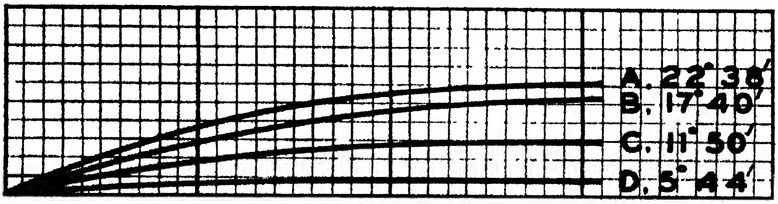

Fig. 6.—Outlines and contact angles of drops of spray oil on water. *A*, Heavy oil (U. R. 95); *B*, light-medium oil (U. R. 92); *C*, light-medium oil (U. R. 80); *D*, light-medium oil (U. R. 92) with 1 per cent glyceryl monoöleate.

A thin film of water without sufficient slope or contour to cause movement of an oil drop placed upon it may be obtained by cleaning to absolute chemical cleanness a strip of glass, such as used in the present investigation to hold in place the strips of leaves. The glass is then covered with distilled water and placed on the slide platform in front of the microprojector objective. A drop of oil may then be placed on the water, upon which it will form a lens (fig. 6). Oils low in U. R. (unsulfonatable residue) and oils containing a surface-active solute such as glyceryl monoöleate were shown to have a lower contact angle on water than pure saturated oil, as figure 6 shows.

Tests with Various Solutes.—Proteins, saponin, soaps, sulfite lyes, alkyl sulfates, alkyl sulfonates, Igepons, petroleum sulfonic acids, and organic acid derivatives were tested with regard to their effect on the contact angle of water on various surfaces. A few preliminary tests resulted in the following values for the contact angle of distilled water alone and distilled water with various solutes on the ventral surfaces of *Viburnum* leaves: water alone, 70° 18′; water with 1 per cent ethyl alcohol, 54° 14′; with 0.01 per cent Vatsol OT, 45° 22′; with 0.1 per cent sodium oleate, 42° 26′; with 0.05 per cent sulfated alcohol or 0.5 per cent dry blood albumin spreader, 38° 50′; with 0.5 per cent Castile soap,

36° 1′; with 0.1 per cent Vatsol OT, 30° 14′; and with 1.0 per cent Vatsol OT, 27° 57′. The greatest reduction in contact angle per unit concentration was obtained with Vatsol OT. On *Viburnum* the compound practically reaches its maximum effect at 0.1 per cent concentration, although on beeswax a reduction in contact angle of 11° 41′ (from 45° 9′ to 33° 28′) was effected by increasing the solute from 0.1 per cent to 1.0 per cent concentration.

While Vatsol OT, 0.1 per cent, proved to be more effective than sodium oleate, 0.1 per cent, in reducing the contact angle of water on *Viburnum* leaves, it was less effective than sodium oleate on a beeswax

TABLE 4

THE RELATIVE SPREADING ABILITY OF SOLUTIONS OF VATSOL OT AND SODIUM OLEATE ON BEESWAX AND *Viburnum* LEAVES AS SHOWN BY CONTACT-ANGLE MEASUREMENTS

Solution*	Substratum	Contact angle
Vatsol OT	Beeswax	43° 19′ ± 31′
Sodium oleate	Beeswax	31° 34′ ± 15′
Vatsol OT	Leaf A	29° 42′ ± 46′
Vatsol OT	Leaf B	30° 14′ ± 40′
Sodium oleate	Leaf A	39° 6′ ± 36′
Sodium oleate	Leaf B	42° 43′ ± 33′

* Concentrations of both solutes were 0.1 gram per 100 cc distilled water. The temperature immediately surrounding the drops was 80° F.

surface, as can be seen in table 4. This interesting observation indicates that chemical relations play a rôle in wetting and spreading as well as do purely physical factors. If only physical factors were involved, it would be expected that the chemical compositions of the solutions or the solid substrata would affect the degree of wetting and spreading as far as they affected free surface energy, but that the difference between the contact angles of two solutions on one substratum would be in the same direction, if not in the same degree, on another substratum. The present test shows that this may not always be the case.

In table 5 are given the contact angles on beeswax surfaces of aqueous solutions with various percentages of seven different wetting and spreading agents. Of special interest in this table is the superior effectiveness of sodium oleate as a wetting agent on beeswax; also, the maximum reduction of contact angle was reached at a concentration of 0.05 per cent or possibly somewhat lower, and from this point on the efficiency of the soap decreased as the concentration increased. The same situation obtains to some extent with regard to the sulfated alcohol 1N–181.

If either red scale or black scale, *Saissetia oleae* (Bern.), are removed

from a leaf and covered with a solution of 0.1 gram Vatsol OT per 100 cc water, the solution will quickly penetrate for varying distances into the tracheae. The extent of penetration of the liquid into the tracheae can be determined because of the change in refractive index of the contents of the tracheal tubes caused by the entrance of the liquid, which makes them appear lighter in color (*4*). The line of demarcation between the air-filled and the liquid-filled portion of a tracheal tube is very distinct.

TABLE 5

CONTACT ANGLES ON BEESWAX OF AQUEOUS SOLUTIONS WITH VARIOUS SOLUTES, AT FIVE DIFFERENT CONCENTRATIONS*

Solute	Per cent concentration				
	0 01	0.05	0.1	0.5	1.0
Vatsol OT†	78° 14′ ± 42′	59° 34′ ± 28′	45° 9′ ± 29′	36° 4′ ± 37′	33° 28′ ± 15′
Vatsol OTC′	81° 12′ ± 36′	80° 15′ ± 24′	75° 28′ ± 54′	63° 7′ ± 42′	50° 27′ ± 20′
Vatsol OT (aqueous)	84° 30′ ± 21′	79° 48′ ± 25′	73° 30′ ± 93′	60° 4′ ± 69′	49° 25′ ± 27′
Tergitol No. 7‡	75° 54′ ± 23′	71° 30′ ± 27′	65° 0′ ± 54′	38° 24′ ± 28′	35° 54′ ± 26′
IN–181§	67° 11′ ± 83′	45° 54′ ± 57′	46° 54′ ± 52′	49° 54′ ± 45′	48° 54′ ± 29′
Areskel¶	80° 24′ ± 74′	71° 23′ ± 35′	59° 54′ ±105′	51° 0′ ± 16′	49° 42′ ± 25′
Sodium oleate	57° 18′ ± 75′	27° 12′ ± 21′	27° 27′ ± 11′	29° 2′ ± 34′	32° 17′ ± 32′

* Each figure is the average of five tests. The temperature immediately surrounding the drop was 80° F.

† Sodium salt of an ester of sulfosuccinic acid.

‡ Sulfated higher alcohol.

§ Sodium lauryl sulfate.

¶ Sodium salt of an alkylated aryl compound.

Wilcoxon and Hartzell (*22*) found that a water solution of 0.5 per cent sodium oleate which penetrated one third to two thirds the length of the tracheae of the larvae of tomato worms (*Phlegethontius quinquemaculata*) was not able to penetrate the tracheae at all after the larvae were killed with HCN. These investigators believed that respiratory movements, or at least vital activity, are necessary to make possible the penetration of this solution into the tracheae. In the present investigation, however, red scale were killed with HCN, and the tracheae of these insects were as readily penetrated by solutions of 0.5 per cent sodium oleate or 0.1 per cent Vatsol OT as were the tracheae of living insects. It is doubtful whether the structurally degenerate red scales have sufficient respiratory or vital activity to influence the penetration of liquids into their tracheae; but, on the other hand, since the tracheal tubes are smaller in diameter than those of the tomato worm, they may be more readily penetrated by means of capillary action, and the liquids may flow a greater distance into the tubes after entering the spiracles.

RELATION OF SURFACE TENSION AND CONTACT ANGLE TO PENETRATION DIFFERENTIAL

Mathematical Considerations.—Hoskins (*13*), neglecting hydrostatic pressure, has expressed the linear rate of flow of a liquid in capillary tubes as:

$$f = \frac{\gamma \cos \theta \, r}{4\eta l}$$

in which γ is the surface tension of the liquid, θ the contact angle, r the radius of the tube, η the viscosity of the liquid, and l the distance penetrated. It should be pointed out that this equation holds only for a condition far from equilibrium; the presence of r in the numerator of the fraction is thus explained.

If there were no more than two variables in the equation above, a differential form of the equation could be integrated to obtain an equation for total length of flow. The variable $\cos \theta$ might be ignored, as Hoskins (*13*) suggests, for situations in which the contact angle is zero or nearly so; but for the purposes of the present investigation reference will be made to the well-known formula for height of capillary rise of a liquid:

$$h = \frac{2\gamma \cos \theta}{rg}$$

An inspection of the formulas given above will show that a reduction in surface tension will invariably decrease the rate or distance of penetration only if the contact angle of the liquid on the inner surface of the capillary tube is zero or sufficiently small so that further decrease in the angle will not result in any important changes in the value $\cos \theta$. If the contact angle of the liquid on the sides of the capillary tube is high, however, as in the case of water in a waxy capillary tube, a decrease in γ results in a decrease in θ and a corresponding increase in $\cos \theta$, so that the value $\gamma \cos \theta$ may be actually increased by reduction in surface tension, with a consequent increase in the rate and distance of penetration of the liquid. An increase in the value $\gamma \cos \theta$ can occur only if the rate of increase of $\cos \theta$ is greater than the rate of decrease of γ. This will later be shown by actual experiment to be what happens.

If an insect, which is wetted with difficulty, is located on rough, porous bark, the spray liquid may penetrate into the bark so rapidly that insufficient liquid remains on the surface to wet the insect, to penetrate into waxy barriers as in the case of the woolly aphid or egg masses, or to penetrate under the bodies of sessile scale insects to the spiracles or regions in which the spray can exert its maximum insecticidal effect. A good example of this disadvantageous penetration differential is the

case of oil sprays applied against the California red scale. The thin film of oil applied to the branches upon which the scales rest is absorbed by the bark so rapidly that it often does not have sufficient time to penetrate the waxy mass (fig. 5) under the scale, which blocks the way of the oil to the spiracles. Consequently, with a given concentration of oil in the spray, nearly all adult red scale may be killed on the leaves, green twigs, and fruit; while perhaps only 60 or 70 per cent may be killed on the larger branches.

Experiments with Water and Aqueous Solutions.—Since the contact angle of water on chemically clean glass is zero, and since glass is transparent, glass tubes offer an excellent means of studying the effect of changes in surface tension on the capillary flow of water. Likewise the inner wall of a glass tube may be coated with a layer of wax so thin as not to interfere greatly with the visibility of the liquid column, yet capable of increasing the contact angle of the liquid to 90°. The same capillary tube may thus serve to demonstrate the effect of changes in surface tension on capillary flow in the one case where contact angle is eliminated as a variable affecting capillary flow and in the other case in which contact angle is an important factor.

Absolute cleanliness is essential in experimental work such as suggested above. On the other hand, if the cleaned glass can be kept in a container with clean air, the matter of air adsorption is probably not of as great importance as many investigators have considered it. Edser (*8*) states that Rayleigh has proved that when glass has been heated in a smokeless flame, water spreads over it readily (implying a zero contact angle), even after the surface has been exposed to pure air for several days. On the other hand, Edser (*8*) states that, ordinarily, greasy matter will be deposited on glass from the air, in which case water will no longer have a zero contact angle on the glass.

In an experiment involving the use of glass capillaries some 1-mm capillary tubing was cut into sections 4 inches long. A mark was made with a file ¼ of an inch from one end of each tube. The tubes were immersed up to this mark in the liquid to be tested. Four tubes were used in each series of tests and these were then cleaned again and used for another series of tests.

All four tubes were placed in a bath of chromic acid and left from one to several hours. From the chromic-acid bath the tubes were placed in a bath of concentrated nitric acid and left for 2 minutes, then rinsed to remove the acid. The tubes were then rinsed in double-distilled water for 2 minutes. They were then placed in a bath of caustic potash for 2 minutes and rinsed in this solution. After this they were again rinsed

in double-distilled water, the water being changed for each successive tube.

The tubes were dried by means of an air stream taken from a pressure line supplied to the laboratory table. The air was first bubbled through chromic acid to remove organic matter, then bubbled through a distilled water bath, and afterwards passed through a bottle loosely filled with glass wool to prevent excess moisture from being carried into the drying towers. The air was passed through two drying towers, the first containing calcium chloride and the second barium perchlorate. Glass wool

TABLE 6

DIFFERENCES IN CAPILLARY FLOW OF WATER IN CLEAN-GLASS AND WAXED-GLASS CAPILLARY TUBES CAUSED BY REDUCTION OF SURFACE TENSION

Inner wall of tube	Concentration of solute (Vatsol OT), per cent	Surface tension of liquid, dynes per cm*	Height of liquid column, cm*
Glass	None	72 7 ± 0.01	2.12 ± 0.09
	0.1	32 1 ± 09	0.85 ± .02
	1 0	29 1 ± 01	0.72 ± .02
Beeswax	None	72 7 ± 01	0.00
	0 1	32.1 ± .09	0.39 ± .03
	1.0	29 1 ± 0 01	0 65 ± 0.02

* Average value for five tests.

was placed at the entrance and exit of each drying tower to prevent the carrying of dust in the air stream from the drying towers.

The air was passed through a glass tube 10 inches long and ¾ inch in diameter. This tube was heated by means of a Fischer burner so that the air might be heated sufficiently to prevent the deposition of any moisture it might contain on the walls of the capillary tubes which were to be dried. A cork containing a hole of just the right size for the capillary tube to fit in firmly was placed at the distal end of the large glass tube and the stream of heated air was passed through the capillary tube to dry it. The air stream was directed through the tube for 3 minutes.

The capillary tube was then placed in a desiccator for 5 minutes to allow it to cool. After this it was placed on a stand constructed so that the tube might be held in place with the file mark at the lower end of the tube directly at the surface of the liquid to be tested. Each tube was left in the liquid 5 minutes before the column of water was measured.

A ring fashioned from a soft metal band was placed around each tube before it was placed in the solution being tested. The column of liquid

in the tube was measured by sliding the metal ring until its bottom edge coincided with the bottom of the liquid meniscus. The tube was then removed from the liquid and the distance from the file mark near the bottom of the tube to the bottom of the metal ring was then measured with a micrometer caliper.

To obtain the wax coating, a capillary tube 16 inches in length and of the same diameter as the tube previously mentioned was used. A 25

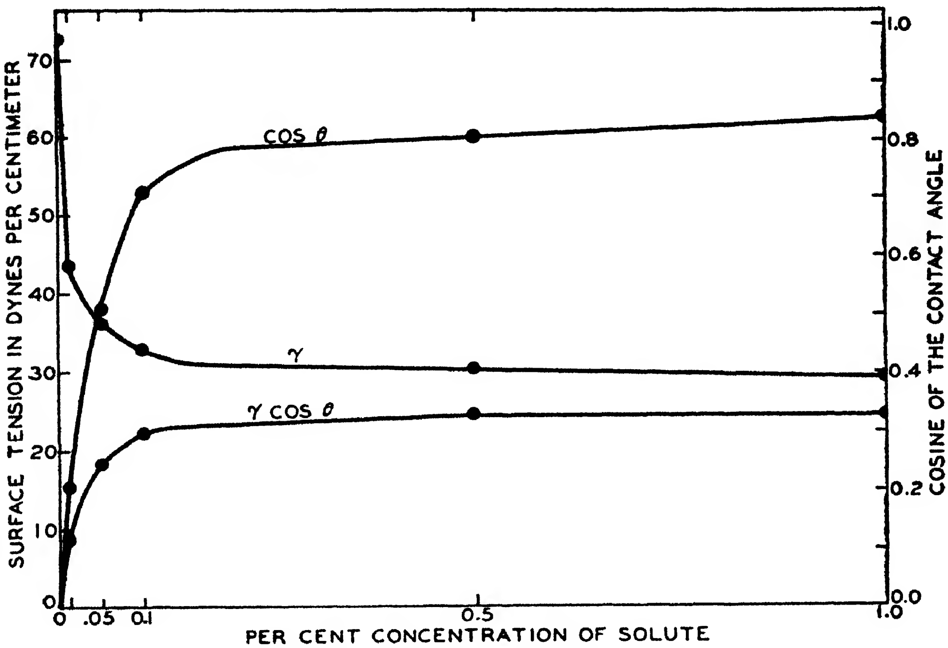

Fig. 7.—Changes in surface tension of water, cosine of the contact angle of water on beeswax, and the product of these two factors, with increasing concentration of Vatsol OT. Compare with curves for capillary flow of liquids shown in figure 8.

per cent solution of beeswax in carbon tetrachloride was drawn into the tube and allowed to drain out. The carbon tetrachloride was evaporated by passing air through the tube. The tube was then cut into four sections 4 inches in length, as before. The height of the column of liquid to be tested was measured in the same way as in the previous tests. Vatsol OT was used as the solute to reduce surface tension, and in the present experiment, as well as subsequent experiments, the solutions were one hour old at the beginning of the tests. However, the flasks containing the solutions were shaken before each test. Determination by means of an Ostwald viscometer showed that in the case of the aqueous solutions the solute had no appreciable effect on viscosity. The experiment was

made at a temperature of 72° F, which was also the temperature of the solutions. The results of the experiment are presented in table 6, and show that a given reduction in surface tension caused an increased penetration in the waxed tubes and a reduced penetration in the clean glass tubes. Thus a penetration differential was effected by the addition of a solute.

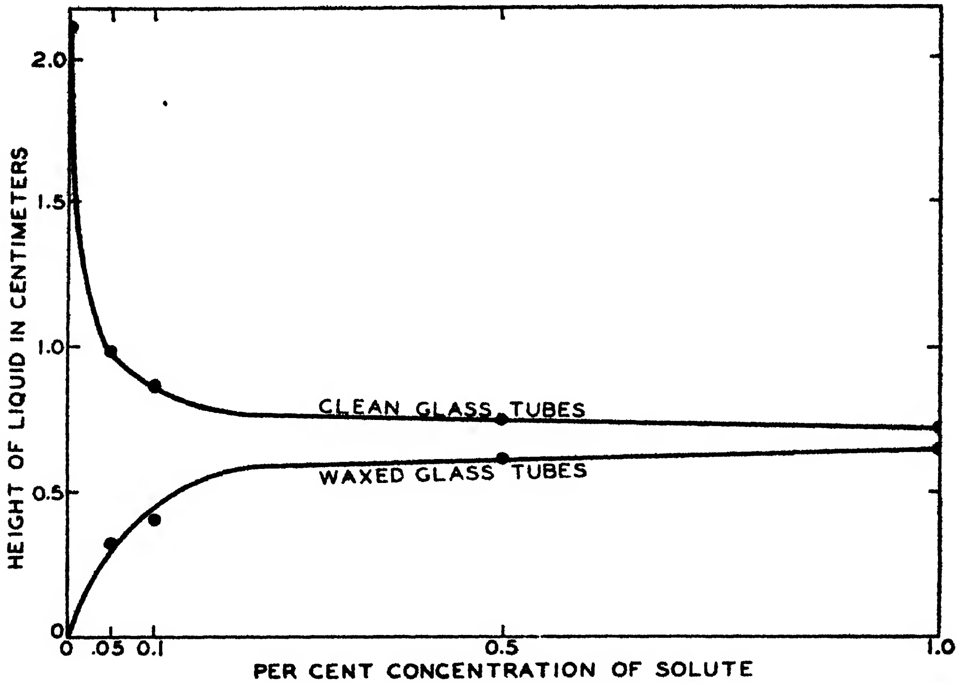

Fig. 8.—Changes in the penetrativity of water in clean glass tubes and waxed glass tubes with increasing concentration of Vatsol OT as determined by experiment. Compare with theoretical expectations as shown in figure 7.

Figure 7 shows graphically the relation of surface tension, the cosine of the contact angle, and the product of these two factors as the concentration of Vatsol OT is increased from 0 to 1.0 per cent. Figure 8, which presents graphically the data in table 6, shows the relation of concentration of solute and, consequently, reduction in surface tension to penetration. The curves for penetration in a clean glass tube and in a waxed tube (fig. 8) are, respectively, rather similar to the curves for surface tension and the product of surface tension and cosine of the contact angle (fig. 7), which is consistent with theoretical expectations.

Data similar to those given above were obtained by using columns of sand as the capillaries. As in all experiments to be mentioned subsequently, the experiment was made at room temperature. Quartz sand of

20-mesh size and less was cleaned by washing in a chromic-acid solution, repeatedly rinsing with distilled water until all traces of chromic acid were removed, and then drying in a flask over a flame and cooling. The sand was then poured into ½-inch glass tubes a foot in length. Another portion of the cleaned sand was immersed in carbon tetrachloride containing 25 per cent paraffin wax. The solvent was evaporated and the sand was sieved through a 20-mesh screen and poured into glass tubes as before. Three tubes with clean sand were placed with their lower ends immersed in water and Vatsol solution: one in pure distilled water, one in a 0.1 per cent Vatsol solution, and one in a 1.0 per cent Vatsol solution. This procedure was repeated with the tubes of waxed sand. For some reason the differences were not so great as those found in connection with the glass capillary experiment (table 6), but they were in the same direction. In the columns of pure sand, in 15 minutes the pure water had risen 10.8 cm; 0.1 per cent Vatsol solution, 9.7 cm; and 1.0 per cent Vatsol solution, 9.2 cm. The corresponding distances of penetration in 16 hours were, respectively, 17.4 cm, 16.1 cm, and 13.7 cm. In the columns of waxed sand in 15 minutes the pure water had not risen a discernible distance, the 0.1 per cent Vatsol solution had risen to a height of 0.4 cm, and the 1.0 per cent solution had risen to a height of 3.1 cm. The corresponding distances in 16 hours were, respectively, 0.2 cm, 0.8 cm, and 3.7 cm.

Some smoothly planed Douglas fir boards were used to test the rate of penetration of water and 1.0 per cent Vatsol solution into wood, on which the contact angle of water is comparatively low. In five separate tests 1 cc of distilled water was placed in a glass cylinder 1 cm in diameter and 4 cm long; this was glued to the board in a vertical position with a commercial waterproof glue, and 4 inches away a similar cylinder was attached in which 1 cc of a 1.0 per cent Vatsol solution was poured. In the five tests, in only one case was the rate of penetration of the Vatsol solution greater than that of the pure water, the average time for the disappearance of the 1 cc of liquid being 55 hours for the pure water and 63 hours for the Vatsol solution.

When the rates of penetration of pure water and a 1.0 per cent Vatsol solution were compared on a board of a commercial pressed fiberboard of Douglas fir wood with a density of 18 pounds per cubic foot, it was found that 3 days were required for the penetration of 1 cc of water and only 20 seconds for the penetration of the Vatsol solution. In this case the penetration was a surface rather than a capillary phenomenon. The layers of wood fiber are so loosely packed that penetration through the mass depends upon the ability of a liquid to spread along a surface.

Since water has an appreciable contact angle on resinous wood, the Vatsol solution spreads much more rapidly on a level surface than pure water and consequently enters a loose mass of wood fibers much more rapidly. This does not mean that the Vatsol solution will enter a more solid mass of wood fibers, in which capillarity is involved, more rapidly, for just the opposite was shown to be the case, as the data for penetration of water into the seasoned Douglas fir lumber show. The effect of surface tension on speed of penetration is then shown to be not only affected by contact angle in the medium penetrated, but is also affected

TABLE 7

Differences in Distances of Flow of Water in Columns of Citrus-Bark Fragments Caused by Reduction of Surface Tension

Concentration of solute (Vatsol OT), per cent	Surface tension, dynes per cm	Condition of bark fragments	Distance (cm) of penetration in:		
			1 hour	4 hours	16 hours
None	72.7	Loosely packed			10.6
1.0	29.1	Loosely packed			8.9
None	72.7	Moderately packed			20.0
1.0	29.1	Moderately packed			13.5
None	72.7	Tightly packed	4.5	9.3	12.0*
1.0	29.1	Tightly packed	1.8	2.0	2.4

* Liquid reached upper limit of column.

by the density of the medium penetrated. If the density of the medium to be penetrated falls below a certain point, the rate of flow may no longer be affected by laws of capillarity, but by the laws of open surface flow in which reduction in surface tension invariably results in more rapid spread of a liquid.

Owing to the great variability in the rate of penetration of water solutions in Douglas fir wood, the method described above was not considered satisfactory as a means of comparing the penetrativity of different solutions. To standardize the capillary medium, fragments of bark were tamped into ½-cm glass tubes in equivalent amounts and in a uniform standard manner and the flow of liquid up into these columns of bark fragments was determined. The bark fragments were made by filing the outer bark off dry orange limbs 2 or 3 inches in diameter. It is this outer bark with which the investigator is concerned in the penetration of films of water or oil when spraying for certain citrus pests.

The results of three tests are summarized in table 7. The data show that in every test a reduction in surface tension of the water retarded

its distance of penetration into the columns of bark fragments. This is to be expected in view of the relatively low contact angle of water on citrus bark. The data likewise show that the more tightly packed the bark fragments are, that is, the more truly the flow of liquid is a capillary phenomenon as opposed to surface-flow phenomena, the greater is the difference in the distance penetrated, between pure water and water with solute. This difference was, however, more definitely emphasized in the great contrast in the ratios of the rates of disappearance of 1-cc portions of water and Vatsol solution in natural Douglas fir wood and the loosely pressed pulp, concerning which the data have been previously given.

The greater penetrativity of pure water than that of Vatsol solution in citrus bark is of special interest since it has been shown that water will not enter under the armor of the California red scale unless its surface tension is greatly reduced. The matted threads of wax (fig. 5) under the body of the red scale make a waxy capillary system, as has been mentioned before. On this wax the contact angle of water is high, whereas on wood it is relatively low. Obviously, reduction in surface tension must reduce the penetration differential of water flowing in wood and scale wax, just as it does in the case of water flowing in glass and wax capillaries (fig. 8).

Experiments with Spray Oil.—Unfortunately it is difficult to reduce the surface tension of spray oil. The surface tension is already so low that very few solutes are sufficiently surface-active in oil to reduce its surface tension more than an unappreciable amount. Pertinent in this regard also are the observations of Langmuir and Harkins (see Clark, *3*, p. 324) that the surface energy of liquids is determined principally by the structure of the surface layer of atomic groupings. The liquid hydrocarbons have methyl groups at the ends of their chains; consequently the surface energies of different compounds are approximately equal and are independent of the length of the chain. Even the alcohols possess about the same surface energy as hydrocarbons because the methyl groups are on the surface, with the polar hydroxyl groups pointing inward.

Glyceryl monoöleate, which is so effective in retarding the penetration of oil into waxy substances such as leaf cuticles because of the polarity given it by its hydroxyl radicals (*7*), has no effect in decreasing the penetration of oil into porous bark because in this case the penetration is a capillary phenomenon, and the glyceryl monoöleate, having no effect on the surface tension or the viscosity of the oil, has no effect on the penetration of the oil into capillary tubes, regardless of the chemical composition of the tubes. This is obvious from an inspection of the

formulas for capillary flow. However, tests were made to demonstrate this point.

In an experiment in which 1-mm glass capillaries were cleaned and coated with beeswax in the same manner as described before with regard to the experiments with aqueous solutions, the height to which columns of oil with and without 1 per cent glyceryl monoöleate ascended in the capillaries was observed and recorded. Each figure given below is the average of five measurements of the distance reached by the column of oil. In the clean glass capillaries, a grade 3 (light-medium) oil rose to a height of 1.03 ± 0.05 cm, and the same oil with 1 per cent glyceryl monoöleate rose to a height of 1.00 ± 0.05 cm; the corresponding figures for the rise of the oils in the waxed capillaries were, respectively, 1.40 ± 0.07 cm, and 1.36 ± 0.03 cm. The addition of glyceryl monoöleate caused no significant difference in the heights of the columns of oil in either the clean glass or the waxed capillaries.

SOME INSECTICIDAL TESTS WITH AQUEOUS SOLUTIONS

The penetration of oil under the body of a red scale (fig. 5) appears to be a prerequisite to lethal effect (*6*). This, in turn, suggested that if toxic aqueous solutions could be made to penetrate under the scale insect's body, they might result in death to the insect either by tracheal penetration, or, if the spiracles were not reached, by penetration of the ventral covering (*6*) of the insect. The possibility of the latter action is enhanced by the fact that once the liquid has penetrated the waxy margin of the insect and is under the body, it is practically unaffected by evaporation. On the exposed portions of an insect an aqueous solution is quickly removed by evaporation.

The contact-angle measurements of aqueous solutions with various solutes served as a valuable guide in the search for liquids with maximum spreading ability on waxy surfaces. Certain organic acid derivatives proved to be especially effective in reducing the contact angle of water on leaves and other waxy surfaces. Water containing these solutes above a certain concentration was able to penetrate the waxy margins of the scales and flow under their bodies. Concentrations of other wetting agents far beyond any amounts that would be practical in commercial usage were not sufficient to produce this effect.

The waxy layer (fig. 5) between the body of a red scale and its substratum (fruit, leaves, bark, etc.) is not homogeneous, but comprises a mass of rather loosely woven threads of wax exuded from the pygidium of the insect (*6*). If the contact angle of an aqueous solution is suffi-

ciently reduced, the solution is able to flow through this mat of waxy threads in much the same manner as oil; yet it has an advantage over oil, for being an aqueous solution it does not have the wax-dissolving properties of oil and consequently does not soak through the waxy cuticle of the leaves, except perhaps to an unappreciable extent. Aqueous solutions may have the spreading properties of oil without the ability of oil to penetrate solid wax. For this reason toxic materials injurious to plants put in aqueous solutions in concentrations sufficient to cause death of insects with safety to the plant would cause serious injury to the plant when put in oil and applied in a dilute oil spray.

In his attempts to find a nicotine spray which would be satisfactory for use against the woolly aphid, *Eriosoma lanigerum* (Hausm.), Greenslade (*12*) had a problem similar to the one encountered in attempting to make aqueous solutions kill red scale. He found that the aphids were killed by a 0.025 per cent concentration of nicotine if the spray liquid actually reached the bodies of the insects, but that it was difficult to wet and penetrate the mass of wax threads which cover each insect and become interlaced over the top of the colony. The effectiveness of the spray against the aphids was so closely correlated with spreading ability that Greenslade used the percentage of kill as a criterion of spreading power. With a 0.025 per cent nicotine solution the per cent kill increased from 1 to 96.6 as the per cent concentration of a soft-soap solution was increased from 0.1 to 3.0 (fig. 9).

The penetration of aqueous solutions under a red scale's body may be demonstrated by coloring the solution with a dye, such as saure fuchsin. Although not enough dye is carried under the insect's body to give a general colored appearance, yet where irregularities of the body occur, such as body segments or fragments of wax, an accumulation of dye occurs and can readily be seen with the aid of the low-power objective of the microscope.

Often when adult red-scale females are dislodged from a fruit, one or more newly emerged larvae ("crawlers") may be seen under the armor in the region of the pygidium. The toxic aqueous solutions which were tried with 0.1 per cent Vatsol OT flowed under the scale armor and killed these crawlers. If the same solution is used with 0.05 per cent Vatsol OT, an occasional crawler may be found alive, while if only 0.01 per cent Vatsol OT is added to the solution, it does not have the ability to spread under the scale armor to any great extent and consequently the majority of crawlers are not reached by the solution and may be seen crawling about actively when the scale is turned on its ventral side and examined with a microscope. As has been stated previously, 0.01 per

cent Vatsol OT has about the same effect in reducing the contact angle of water on a leaf surface as 0.5 per cent sulfated alcohol or 0.5 per cent sodium oleate. A solution may give a complete wetting of a surface without having the ability to penetrate under the scale armor. For example, if twice as much blood albumin spreader as is ordinarily used in commercial spraying (8 ounces per 100 gallons of water) is placed in an

TABLE 8

THE INFLUENCE OF THE WETTING AGENT ON THE EFFICACY OF AQUEOUS SOLUTIONS OF SODIUM DINITRO-O-CYCLOHEXYLPHENATE AGAINST CALIFORNIA RED SCALE

Test no.	Concentration of toxicant, per cent	Wetting agent	Concentration of wetting agent, per cent	Number of insects counted*	Net mortality,† per cent
1	0.1	Vatsol OT	0.025	2,141	21.2 ± 0 73
2	0 1	Vatsol OT	0.05	1,872	57.3 ± 0.94
3	0 1	Vatsol OT	0 1	3,306	73.6 ± 0 53
4	0 1‡	Vatsol OT	0.1	2,348	83 2 ± 0 63
5	0 1	Vatsol OT	1.0	3,447	80.7 ± 0.55
6	0.2	Vatsol OT	1 0	2,313	84 8 ± 0 61
7	0 5	Vatsol OT	1 0	1,783	99.5 ± 0.14
8	0 1	Tergitol	0.1	3,416	32 7 ± 0 67
9	0.05	Tergitol	1.0	2,731	69 2 ± 0 73
10	0.1	Tergitol	1 0	2,249	78.6 ± 0 84
11	0 1	Blood albumin	0 03	1,237	0.0
12	0 1	Blood albumin	0 3	1,825	46 6 ± 0 96
13	0 1	None	.	946	0 0
14	0 0	Vatsol OT	1 0	1,362	0.0
15	0 0	Tergitol	1.0	1,573	0 0

* There were 4,741 insects in the control, of which 66.2 per cent were alive.

† Calculated as $\frac{x-y}{x}$ when x = percentage left alive in untreated lot and y = percentage left alive in treated lot. Probable error computed from the formula $P.E. = 0.6745\sqrt{\frac{pq}{n-d}}$, when p = net per cent mortality; q = 100 − net per cent mortality; n = number of insects counted; and d = number of n which would have died from natural causes during the course of the experiment as determined by the percentage of dead insects in the check.

‡ The toxicant was dissolved in acetone and the acetone solution was used at 1.0 per cent concentration.

aqueous solution sufficiently toxic to kill the crawlers which happen to be located under the armor of a scale, none of the crawlers will be killed by the solution, even though a uniform wetting of the surface of the scale-infested fruit is obtained. If a more effective spreading agent is added to the solution containing the blood albumin, it will flow under the scale armor and kill the crawlers and the adult insect also, provided the aqueous solution is sufficiently toxic.

Data on the insecticidal effect of a compound known as dinitro-o-cyclohexylphenol were first published by Kagy and Richardson (*14*), who dissolved the compound in oil. In the present investigation, dinitro-o-cyclohexylphenol was used in aqueous solutions in the form of a so-

dium salt. Kagy[9] had already determined the merits of this particular salt of dinitro-o-cyclohexylphenol as well as other salts of the same compound as early as 1931.

The test insects were California red scale infesting grapefruit. Counts were made on adult and gray-adult (*6*) insects only. The scale-infested fruits were dipped in the toxic solutions and kept at a temperature of

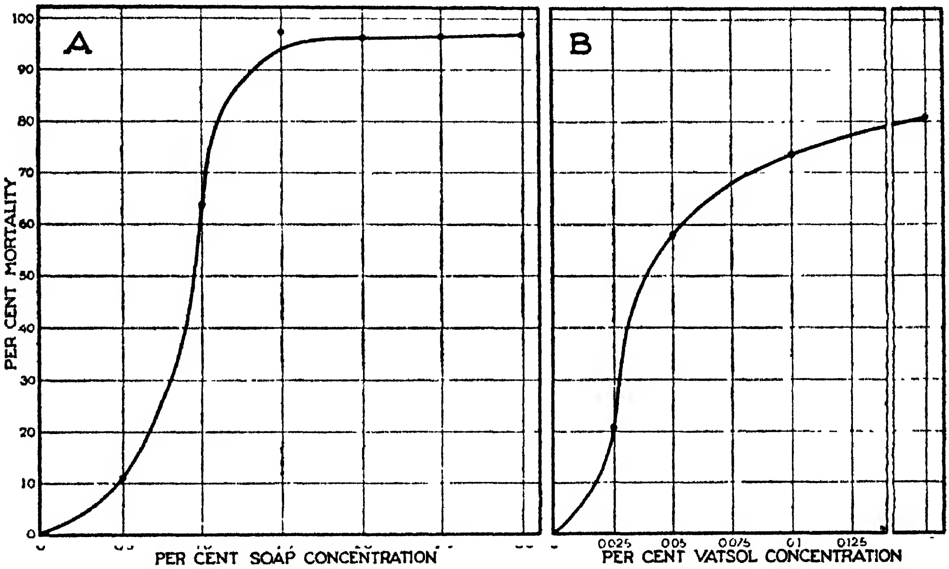

Fig. 9.—The effect of the addition of spreader to contact sprays on their effectiveness against insects protected by waxy barriers. *A*, Mortality curve of woolly aphid with increasing concentrations of soap in a nicotine solution (after Greenslade). *B*, Mortality curve of red scale with increasing concentrations of Vatsol OT in a sodium dinitro-o-cyclohexylphenate solution.

67° F and a relative humidity of 70 per cent for 20 days before being examined. The results of the experiment are tabulated in table 8. Insect mortality was rather closely correlated with the spreading ability of the solution.

In test no. 4, dissolving the toxicant in acetone caused it to be dissolved in the aqueous solution more rapidly and completely when the acetone solution was poured into the water than if the toxicant had been added directly to the water without an intermediary solvent. This was reflected in the improved kill as compared to test no. 3. In test no. 11, the amount of dry blood albumin was about in the proportion in which it is used commercially in the tank-mixture method of using oil spray.

[9] Kagy, J. F. 1931. Report of Crop Protection Institute Investigation, Dow Chemical Company Fellowship. (Unpublished manuscript.) 81 p. On file with the Crop Protection Institute, Durham, N. H.

Figure 9 shows the relation of the insecticidal efficiency of two toxic aqueous solutions to the spreading ability of the solutions as determined by the concentration of the spreader. Curve *A* depicts the increase in mortality of the woolly apple aphid, *Eriosoma lanigerum* (Hausm.), from a 0.025 per cent nicotine solution as the per cent of soft-soap solution was increased from 0.1 to 3.0 (*12*). The increase in mortality of California red scale, *Aonidiella aurantii* (Mask.), from 0.1 per cent solutions of sodium dinitro-o-cyclohexylphenate as the concentration of Vatsol OT was increased from 0.025 to 1.0 per cent is shown in *B*. In either case the limiting factor in the use of the solutions as far as insecticidal efficiency is concerned, is not the toxicity *per se* of the solutions, but the ability of the solutions to penetrate the mat of waxy threads covering the aphids in one case and the waxy capillary system beneath the bodies of the scales in the other.

The sodium dinitro-o-cyclohexylphenate solutions were used in the present experiment only to show the relation of spreading to insecticidal efficiency; the writer does not wish to imply that the toxicant involved shows promise as a practical insecticide for use on citrus trees in the way it was used in the present experiment. As far as is known to date, the phytocidal effect of the compound limits its use in this regard.

SUMMARY

Although the static contact angle may not be a true index of the wetting and spreading properties of a liquid under the dynamic conditions which obtain during the actual spraying operation, it indicates the spreading and penetrating qualities of the liquid after it has been deposited on the plant surface.

An attempt was made to devise a means of contact-angle measurement which would combine practicability and accuracy. Previous methods of contact-angle measurement have been based on the projection or reflection of the image of a solid face immersed in a liquid in such a way that the angle made by the water/air surface with that of the water/solid is at the minimum value of the contact angle, or by the projection of photographic negatives made of drops of liquids resting on solids. Measurements of the contact angle liquid/solid are then made by means of a protractor on the projected or reflected image.

The present method involves the projection of a drop of liquid resting on a perfectly horizontal substratum and the tracing of the projected image. A microprojector built for material mounted on microscope slides was adapted, after minor adjustments, for the purpose of projecting liquid drop outlines.

The outlines of drops 5 mm in diameter were found to be elliptical, but were practically circular for liquids with contact angles less than 65°. Smaller drops retain a spherical shape at even higher contact angles. The contact angles may be derived by differentiation of the equation of the drop outline. If the drop is a segment of a sphere, a more simple method may be employed for calculation of the contact angle.

It was shown statistically that five measurements of the contact angle of a given liquid are sufficient to establish a highly significant mean and that a difference as low as 3° 32′ can be reliably determined. Size of drop had no significant effect on contact angle.

The nature of the substratum was found to have a great influence on the contact angle liquid/solid. Thus, leaves of different plant species, leaves of different ages on the same plant, and different sides or portions of a single leaf cause variation in the contact angle of a given liquid. A great difference in the contact angle of oil on leaf wax and oil on the wax of scale insects was found, the former being the greater.

Various solutes were tested with reference to their effect in lowering the contact angle of water and oil on various solids. The relative spreading ability of solutions on one substratum does not necessarily indicate their relative spreading ability on other substrata. Thus Vatsol OT was found to be a more effective spreader per unit concentration than sodium oleate on *Viburnum* leaves but less effective on beeswax.

According to the formula for rate and distance of penetration of liquids in capillary tubes, a reduction of the surface tension and consequently of the contact angle of a liquid may increase its penetrativity into tubes on the inner surface of which it has a high contact angle, but reduces its penetrativity in tubes on the inner surface of which it has a very low or zero contact angle. This principle was experimentally verified with aqueous solutions in chemically clean glass tubes as contrasted to waxed glass tubes. The practical application of the principle is that by a reduction of surface tension the rate of penetration of aqueous solutions into porous solids such as bark, on which the solutions have a very low contact angle, may be decreased and their rate of penetration through the waxy threads exuded by insects, or under their bodies and into their spiracles, may be increased. Consequently the usual great differences in the rate of penetration of the solutions into insects and their substrata, if they happen to be located on porous bark, is reduced.

The surface tension of water may be sufficiently reduced by the addition of some of the more effective wetting agents so that toxic aqueous solutions can penetrate under the body of the red scale, *Aonidiella aurantii* (Mask.), and cause the death of the insect. The percentage of

mortality of red scale from a given toxicant was found to be correlated with the effectiveness and concentration of the spreading agent; the more effective spreading agents and the higher concentrations resulted in greater insecticidal efficiency of the solution. The penetration of aqueous solutions under the body of the red scale may be demonstrated with the aid of a water-soluble dye.

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